Discussion of "Sediment characteristics regulate anaerobic oxidation of methane coupled with nitrate and nitrite in the hyporheic zone"

Responses to the Comments from the Reviewer 2

Note: The reviewers' comments are shown in black, and our responses to the comments are shown in blue.

General answer

We sincerely thank Reviewer 2 for the thorough and constructive assessment of our manuscript and for recognizing the importance of the research question, the value of our dataset, and the integrative approach combining porewater hydrochemistry, stable isotope analyses, microbial community data, and reactive transport modelling.

The reviewer emphasized that some mechanistic and causal interpretations should be more closely aligned with the level of support provided by the available evidence. In particular, the reviewer recommended a clearer distinction between direct observations and model-based interpretations, stronger statistical and uncertainty analyses, improved statistical and quantitative reporting, and revision of the writing for greater clarity.

Following the reviewer's recommendations and taking the comments of Reviewer 1 into account, we will comprehensively revise the manuscript to address these concerns. We will critically reassess the interpretations and conclusions to ensure that they are supported by the available evidence and more clearly distinguish direct observations from model-based interpretations. We will strengthen the statistical and uncertainty analyses and the quantitative reporting of the results, improve the description and presentation of model parameterization and sensitivity analysis and incorporate the relevant information into the main text, restructure the manuscript by separating the Results and Discussion to improve the presentation of the study findings, and thoroughly revise the text for greater clarity and consistency. We believe that these revisions will substantially improve the scientific rigor, transparency, and readability of the manuscript.

Specific comments

1. PROFILE and PHREEQC are shown as jointly supporting the mechanistic conclusion, but the pathway-specific result comes only from PHREEQC. PROFILE identifies net electron-acceptor source–sink zones and cannot distinguish among heterotrophic denitrification, NO_3^--DAMO, NO_2^--DAMO, or overlapping processes. The models also do not appear to exchange parameters or constrain one another. They should therefore be described as parallel analyses rather than an integrated modeling framework, and the conclusion that AOM (particularly NO_2^--DAMO) dominates methane removal should be attributed explicitly to PHREEQC. This issue is present in multiple places in the paper. Example: "By integrating porewater chemistry, microbial community analysis, and inverse reactive transport modeling, this study further demonstrates that AOM represents the dominant methane sink..." attributes the dominance of AOM to the integration of porewater chemistry, microbial data, and inverse reactive transport modeling, but the pathway partition actually comes from the PHREEQC forward model, not from PROFILE or the integration itself.

   Thank you for this important comment. We agree that the respective functions of PROFILE and PHREEQC and the relationship between the two modelling approaches were not described with sufficient precision in the original manuscript. As discussed in detail in our responses to Reviewer 1, Comments 1 and 2 on modelling, we have reassessed the role and applicability of PROFILE and will ultimately remove the PROFILE modelling and its corresponding results and interpretations from the revised manuscript.

   The revised modelling section will therefore focus on PHREEQC. The PHREEQC reaction scenarios are formulated based on the measured hydrochemical profiles, stable isotope signatures of methane, and microbial community data. The quantitative evaluation of the plausible contributions of the different biological methane oxidation pathways will be derived specifically from the PHREEQC simulations. We will revise the relevant statements throughout the manuscript, including the example identified by the

reviewer, to attribute these pathway-specific quantitative results explicitly to PHREEQC and adjust the corresponding interpretations according to the level of support provided by the revised modelling results.

2. The >99% NO_2^--DAMO result relies on parameter choices that are largely placed in the Supplement and are not tested with a sensitivity analysis. Because NO_2^- was never measured above detection and the model was designed to keep it near or below the detection limit, using its non-detection as support for the modeled mechanism is partly circular. The authors should show in the main text whether NO_2^--DAMO remains dominant across plausible ranges of μmax, Ks, fa, X, Y, and background electron-acceptor decay. If the pathway split is not robust, the >99% value should not be presented as a firm result; the mechanism should instead be framed throughout as one plausible scenario consistent with the data, with that uncertainty stated at first mention rather than only at the end.

   Thank you for this important comment. We agree that nitrite concentrations below the analytical detection limit should not be interpreted as independent evidence for rapid nitrite turnover and subsequently used to support the model interpretation. We also agree that the robustness of the inferred methane oxidation pathway partitioning should be evaluated across plausible parameter ranges rather than based on a single parameterization.

   To address these concerns, we will substantially revise the modelling framework by considering alternative conceptual scenarios for nitrate/nitrite transformation and by performing uncertainty and sensitivity analyses (UASA) within plausible parameter ranges. In the revised framework, nitrite concentrations below the analytical detection limit will be used solely as an observational constraint on the simulated nitrite concentrations, rather than as independent evidence for rapid nitrite turnover or a specific nitrogen transformation pathway. The UASA will assess how uncertainty in the key parameters affects the inferred contributions of the different methane oxidation pathways and whether the resulting pathway partitioning remains robust across the investigated parameter ranges. Accordingly, we will no longer present the >99% contribution of NO_2^--DAMO obtained from a single parameterization as a firm result. Instead, the interpretation and level of certainty assigned to the relative pathway contributions will be based on their robustness across the complete set of simulations. The associated uncertainty will be stated when the modelling results are first presented and reflected consistently throughout the revised manuscript. The rationale and implementation of these revisions will be described in detail in our responses to Reviewer 1, Comments 3 and 4 on modelling.

3. PROFILE assumes steady state, yet the interpretation of profiles 4 and 5 brings up seasonal displacement of redox and methane-oxidation zones. This does not necessarily invalidate the model, because each profile could represent a different quasi-steady seasonal state, but that assumption is not demonstrated. The authors should justify that porewater gradients were sufficiently stable over the peeper equilibration period or acknowledge that transient concentration changes may be incorporated into the inferred reaction terms, limiting quantitative interpretation of reaction intensities.

   Thank you for this important comment. We agree that the steady-state assumption of PROFILE introduces additional uncertainty when the temporal stability of the measured porewater gradients over the peeper equilibration period cannot be demonstrated. Under these conditions, transient changes may influence the measured concentration profiles and consequently limit the quantitative interpretation of the source–sink terms inferred by PROFILE under the steady-state assumption.

   As discussed in detail in our responses to Reviewer 1, Comments 1 and 2 on modelling, we have reassessed the applicability and contribution of PROFILE in light of its underlying assumptions and the objectives of the present study. Considering these limitations together, we have ultimately decided to remove the PROFILE modelling and all corresponding results and interpretations from the revised manuscript.

4. The B–C comparison directly supports a permeability effect, but not an independent OC effect. Bulk OC measured by LOI was broadly similar between the two sites, while OC availability and reactivity were not measured. The authors should present permeability as the demonstrated control and treat OC availability/reactivity as a hypothesis rather than a driver. The abstract and conclusion should be revised accordingly.

   We thank the reviewer for this valuable comment. We agree that the comparison between locations B and C supports an association between sediment permeability and the observed differences, whereas it does

not provide independent evidence for an effect of organic carbon availability or reactivity. Because bulk organic carbon contents (estimated by LOI) were broadly similar between the two locations, while organic carbon availability and reactivity were not measured directly, we will revise the manuscript accordingly. Throughout the revised manuscript, the association between sediment permeability and the observed differences will be emphasized based on the available observations, whereas the potential role of organic carbon availability and reactivity will be discussed explicitly as a hypothesis rather than as a demonstrated driver. Corresponding revisions will also be made in the Abstract and Conclusions.

5. There is repeated equating of undetectable methane with non-methanogenic conditions, but the data only demonstrate an absence of methane accumulation. Methane could still be produced and removed through oxidation, advection, or ebullition before accumulating to detectable levels. The authors should replace "non-methanogenic" with "no detectable methane accumulation" and explicitly acknowledge that methanogenesis cannot be excluded without direct rate measurements.

   Thank you for this valuable comment. We agree that the absence of detectable methane should not be equated with the absence of methanogenesis. We will revise the manuscript to replace terms such as "non-methanogenic conditions" with "no detectable methane accumulation" where appropriate. We will also explicitly acknowledge that methane may have been produced but removed through oxidation or transport before accumulating to detectable concentrations, and that methanogenesis cannot be excluded without direct rate measurements.

6. Methane supersaturation and probable free-gas formation are identified but their consequences into the methane budget or PHREEQC model is not shown. Ebullition could export methane directly past the shallow oxidation zone, so modeled dissolved-phase oxidation cannot be interpreted as the complete methane fate. The authors should discuss and, where possible, constrain the ebullitive flux, and clarify whether PHREEQC represents gas–water partitioning. If it does not, they should explain how supersaturated CH_4 measurements were treated and acknowledge this limitation.

   We thank the reviewer for this valuable comment. We agree that ebullition may represent an important methane transport pathway under methane-supersaturated conditions and could bypass the shallow oxidation zone. However, ebullitive fluxes were not measured in this study and therefore cannot be constrained quantitatively.

   The PHREEQC model considers only dissolved-phase transport and reactions and does not explicitly simulate gas–water partitioning or ebullition. Accordingly, the measured dissolved methane concentrations, were used directly as observational constraints for model calibration without representing the formation or transport of a free-gas phase. We will clarify this modelling assumption in the revised manuscript and explicitly state that the simulated methane oxidation represents the modeled fate of dissolved methane only and should not be interpreted as a complete methane mass balance under conditions where free-gas formation and ebullition may occur. We will also discuss this limitation and its implications for the overall methane budget in the Discussion.

7. Sulfate reduction is inferred from declining SO_4^{2-} concentrations, but the same conservative-tracer check used for nitrate is not applied on it. The authors should examine SO_4^{2-} relative to Cl^- to distinguish reactive sulfate loss from dilution or transport effects. They should also report the number and statistical support of the PROFILE reaction zones and present reoxidation of unmeasured HS^-/H_2S as a hypothesis rather than a demonstrated explanation for the alternating sulfate source–sink pattern.

   We thank the reviewer for this valuable suggestion. We agree that declining SO_4^{2-} concentrations alone do not provide sufficient evidence for microbial sulfate reduction, as conservative mixing or dilution may contribute to the observed concentration patterns. As described in detail in our response to Reviewer 1, Comment 4 on microbiology, we will therefore calculate SO_4^{2-}/Cl^- ratios for all porewater profiles to evaluate the potential influence of conservative mixing. We will also revise the manuscript so that sulfate reduction and sulfide reoxidation are discussed only as plausible hypotheses where direct supporting evidence is lacking.

   Regarding the requested characterization of the PROFILE reaction zones, as discussed in our responses to Reviewer 1, Comments 1 and 2 on modelling, we have reassessed the applicability and contribution of PROFILE and ultimately decided to remove the PROFILE modelling from the revised manuscript. Consequently, the PROFILE-derived reaction zones, including the alternating sulfate source–sink terms

and their corresponding interpretations, will also be removed and their number and statistical support will therefore no longer be reported.

8. The conclusion that spatial heterogeneity outweighs temporal variability is not fully supported by the sampling design. The only methanogenic site, C, was sampled only twice in cool-season months, and location A was sampled once, so spatial and seasonal effects cannot be separated reliably. The authors should instead state that spatial heterogeneity appears to be an important control, while acknowledging that the current design cannot determine whether it is stronger than temporal variability. A summer profile at site C would be needed to support the stronger claim.

   We thank the reviewer for this thoughtful comment and agree that our original wording overstated the level of inference that can be drawn from the current sampling design. Our intention was to emphasize that the observed spatial pattern remained consistent across the available sampling campaigns rather than to quantitatively compare the relative magnitude of spatial and temporal variability.

   In the present study, although repeated sampling at locations B, C, and D showed temporal variations in the hydrochemical profiles, the presence or absence of methane accumulation at each location was consistent across sampling periods. Based on the available observations, the spatial pattern appeared to remain consistent throughout the investigated sampling period. However, we agree that the current sampling design, particularly the limited seasonal coverage of location C and the single sampling campaign at location A, does not permit a rigorous assessment of whether spatial variability is greater than temporal variability. Accordingly, we will revise the corresponding statements throughout the manuscript to remove this stronger conclusion. The revised manuscript will state that the present dataset does not allow a rigorous comparison of the relative importance of spatial and temporal variability.

9. The microbial conclusions rely largely on visual patterns in relative-abundance data without statistical support. The authors should test community differences using compositionally appropriate methods, such as CLR/Aitchison-based PERMANOVA, use PERMDISP specifically for dispersion, and report variability among biological replicates. They should also apply the "abundance does not show activity" caveat consistently: enrichment of methanogens, sulfate reducers, or *Methylomirabilis* indicates potential, not active methanogenesis, sulfate reduction, or NO_2^--DAMO without independent functional evidence.

   We thank the reviewer for this important comment. We agree that the interpretation of microbial community patterns should not rely primarily on visual differences in relative abundance or ordination, and that differences in overall community composition and dispersion require appropriate statistical support.

   Following the reviewer's suggestion, we will apply appropriate zero replacement and CLR transformation to the OTU count table and calculate Aitchison distances to compare overall microbial community composition. The resulting Aitchison distance matrix will be used consistently for the NMDS ordination and subsequent statistical analyses. PERMANOVA will be applied to evaluate differences in overall microbial community composition between Locations B and C, while PERMDISP will be used specifically to test differences in multivariate community dispersion. Thus, the patterns visualized by the NMDS ordination will be evaluated statistically rather than interpreted from the visual spread of samples alone.

   Variability between replicates is already visualized in Fig.3a and Fig.5, where the two replicate relative-abundance profiles at each sampled depth are displayed individually. We will clarify this more explicitly in the corresponding figure captions. In the revised ordination and statistical analyses, replicate samples will likewise be retained individually throughout the NMDS, PERMANOVA, and PERMDISP analyses.

   We further agree that taxonomic abundance does not demonstrate metabolic activity. Therefore, throughout the revised manuscript we will interpret the presence or enrichment of methanogens, sulfate reducers, and *Candidatus Methylomirabilis* as indicating the potential for the corresponding metabolic processes. Statements implying active methanogenesis, sulfate reduction, or NO_2^--dependent anaerobic methane oxidation will be removed or rephrased unless supported by the available evidence.

10. Permeability is treated as the main control on redox zonation, yet it was estimated indirectly from sieve analysis rather than measured in situ. Because partial re-clogging can substantially reduce hydraulic conductivity without being captured by grain-size estimates, the reported values may not represent

conditions during sampling. The authors should report the empirical estimation method and uncertainty, validate it with direct hydraulic measurements where possible, and test how uncertainty in permeability, velocity, and dispersion affects the PHREEQC pathway partition.

We thank the reviewer for this important comment. We agree that the permeability values were estimated indirectly from sediment grain-size distributions rather than measured in situ and therefore may not fully represent the hydraulic conditions during sampling.

In the revised manuscript, we will describe the empirical estimation method more clearly and explicitly acknowledge its limitations. Hydraulic conductivity was first estimated empirically from the sediment grain-size distributions, and permeability was subsequently calculated from the estimated hydraulic conductivity. These estimates therefore do not account for temporal changes in hydraulic properties caused by processes such as partial re-clogging after the fines-removal treatment. Consequently, the estimated hydraulic conductivity and derived permeability may differ from the effective field conditions during sampling.

We will also explicitly discuss the applicability of the empirical relationship used to estimate hydraulic conductivity in this study. In particular, the 0–16 cm sediment layer at location B consists almost entirely of coarse gravel and therefore lies outside the original calibration range of the empirical equation. As indicated in the revised Supplementary Table S4, the calculated uniformity coefficient (Cu=1.55) is below the recommended applicability range. Because Cu = 1.55 falls outside this range, we applied the correction factor corresponding to Cu = 5 as a lower-bound approximation. Since the correction factor increases with decreasing Cu, this approximation may underestimate the hydraulic conductivity of this layer. This uncertainty is consequently propagated to the derived permeability. This limitation will be explicitly acknowledged in the revised manuscript.

Because direct hydraulic measurements were not conducted during the sampling period, retrospective validation against field-based hydraulic measurements is not possible. We will therefore explicitly acknowledge this uncertainty when discussing the association between permeability and hydrochemical zonation and biogeochemical processes and avoid overinterpreting the estimated values.

Finally, we will evaluate the influence of this uncertainty through an uncertainty and sensitivity analysis (UASA) of the PHREEQC model. Plausible ranges of permeability, advective velocity, and dispersion will be examined to assess whether reasonable variations in transport parameters alter the simulated hydrochemical profiles or the inferred partitioning among methane oxidation pathways.

11. The title and abstract present causation and pathway identity as established, although the evidence is observational and the nitrate/nitrite-coupled AOM mechanism comes primarily from modeling at one methanogenic site. Terms such as “regulate,” “selected for,” and definitive statements about OC availability and AOM mechanism should be softened. The authors should describe sediment characteristics as associated with, or likely influencing, redox zonation, and present NO_3^--/NO_2^--coupled AOM as a modeled plausible mechanism rather than a demonstrated process. The limited seasonal and spatial coverage of the methanogenic site should also be acknowledged in the abstract.

We thank the reviewer for this valuable comment. We agree that the original title, abstract, and several statements throughout the manuscript conveyed a level of certainty that exceeded the evidence provided by the available observations and model results.

To address this concern, we will revise the title, abstract, and manuscript to more clearly distinguish between observational evidence, model-supported process interpretations, and mechanistic hypotheses. The revised title will better reflect that the objective of this study is to improve process-level understanding rather than to demonstrate causal relationships or uniquely identify methane transformation pathways. Terms implying direct causation, such as “regulate” and “selected for”, as well as definitive statements regarding OC availability, will be replaced with more cautious wording reflecting associations or potential influences supported by the available observations.

Interpretations regarding the potential influence of sediment characteristics on transport processes, hydrochemical gradients, microbial community distribution, and methane-related biogeochemical processes will likewise be presented cautiously based on the available evidence, rather than as directly demonstrated causal relationships. Statements regarding specific methane oxidation pathways will be

revised after completion of the revised PHREEQC simulations, according to the level of support provided by the resulting model outcomes.

We also agree that the spatial and temporal scope of the study should be communicated more clearly. Accordingly, the revised abstract will explicitly acknowledge the limited spatial and temporal coverage of the investigated methanogenic conditions. The resulting process-level interpretations will therefore be presented as a case study aimed at improving process understanding, rather than as universally applicable conclusions for river hyporheic zones.

12. Permeability is treated like an isolated causal driver, but the cross-site comparison is partly observational and confounded by gravel size, restoration history, OC characteristics, and sampling season. Only the fines-removal treatment at location B provides a direct causal test. The authors should foreground that manipulation, replace broad terms such as "determine," "demonstrate," and "primary control" with association-based language for A/B/C/D comparisons, and explicitly acknowledge the major site-level confounds.

We thank the reviewer for this thoughtful comment. We agree that the manuscript did not sufficiently distinguish between the experimental manipulation at Location B and the observational cross-site comparisons among Locations A–D. In the revised manuscript, we will explicitly distinguish between these two components of the study design. The fine sediment removal at Location B will be presented as the only direct experimental manipulation, whereas cross-site comparisons among Locations A–D will be interpreted as observational evidence and discussed using association-based rather than causal language. Accordingly, throughout the revised manuscript, expressions such as "determine", "demonstrate", and "primary control" will be replaced by more cautious wording (e.g., "associated with", "consistent with", or "may influence") where appropriate. We will also explicitly acknowledge that gravel size, restoration history, organic carbon characteristics, sampling season, and other site-specific environmental factors may all contribute to the observed differences and therefore represent potential confounding factors in the observational comparisons.

13. There is no clear distinction between spatial replication from repeated sampling. The study includes one channel, four locations, and only one methanogenic site, while several of the eight profiles are temporal repeats from the same location. The authors should report this effective replication explicitly, avoid treating repeated profiles as independent sites, and limit conclusions to this study system. The global methane-budget discussion is appropriate as motivation, but this dataset does not support quantitative generalization to riverine sediments broadly.

We thank the reviewer for this helpful comment. We would like to clarify that the distinction between spatial sampling locations and repeated temporal observations is already described throughout the manuscript. Specifically, the Methods describe four spatially distinct sampling locations (A–D), while Fig. 2 labels each hydrochemical profile by its corresponding location and sampling date (Location A: Profile 1; Location B: Profiles 2–3; Location C: Profiles 4–5; Location D: Profiles 6–8). This distinction is also maintained in Section 3.1, where the locations are explicitly discussed together with their corresponding profile numbers. In the Results and Discussion, repeated profiles are consistently interpreted as temporal observations from the same location rather than as independent sampling sites.

To avoid any potential ambiguity, we will revise the summary paragraph to explicitly state that the study comprises four spatial sampling locations within a single stream, and that three of these locations (B, C, and D) were sampled repeatedly over time, yielding Profiles 2–8. We will also revise the Discussion and Conclusions to further emphasize that repeated profiles are not treated as independent sampling sites and that our interpretations are restricted to the investigated stream system rather than generalized to riverine sediments more broadly.

14. The Introduction presents atmospheric methane emissions and the global CH_4 budget as the central problem, but the study measures pore-water concentrations and modeled internal methane cycling rather than methane flux to the atmosphere. The authors should distinguish controls on methane production, oxidation, and accumulation from controls on actual emissions. Global-emissions framing is appropriate as motivation, but the conclusions should be narrowed to the potential for emission inferred from subsurface zonation unless direct flux measurements are available.

Thank you for this insightful comment. We agree with the reviewer's observation that our study primarily focuses on pore-water concentrations and internal methane cycling (production, oxidation, transport, and accumulation) at the pore scale, rather than direct atmospheric methane flux measurements.

We agree that providing a global context is essential to demonstrate the scientific relevance of our study at the pore-scale. As discussed in the manuscript, the internal methane cycling processes occurring within the hyporheic zone represent the subsurface processes that may ultimately influence methane emissions to the atmosphere. Therefore, understanding these subsurface processes provides important process-level context for assessing the potential for methane emission.

To better reflect the scope of our study, we will revise the Introduction, Discussion and Conclusions to more clearly distinguish controls on internal methane cycling from controls on actual atmospheric methane emissions. Accordingly, our conclusions will be restricted to the dissolved methane-related biogeochemical processes occurring within the hyporheic zone and to the methane oxidation potential. These findings may provide process-level insights into factors that could indirectly influence atmospheric methane emissions, but they should not be interpreted as direct evidence of methane emissions or directly linked to atmospheric methane fluxes.

15. "thereby providing clear evidence of active microbial methane oxidation.": The isotope enrichment (line 364, ~4.8‰) is good evidence of oxidation but it doesn't distinguish aerobic MOx from anaerobic AOM (both fractionate). Calling co-located methanotroph abundance "compelling evidence for active MOx" overstates, because the same section's own model concludes the oxidation is >99% anaerobic (NO_2^--DAMO), not MOx. So "compelling evidence for active MOx"(line 569) is in conflict with >99% anaerobic claim. Recommend: let the model adjudicate aerobic vs anaerobic.

We thank the reviewer for this valuable comment. We agree that the observed enrichment in $\delta^{13}C$-CH_4 provides evidence for methane oxidation but does not, by itself, distinguish between aerobic and anaerobic methane oxidation. We will revise the corresponding discussion accordingly.

We also agree that the presence of taxa associated with aerobic MOx inferred from 16S rRNA gene sequencing should not be interpreted as direct evidence of active MOx. As discussed in detail in our response to Reviewer 1, Comment 3 on microbiology, we will revise the microbial and modelling interpretations to resolve this inconsistency. Microbial community data will be interpreted as indicating the potential presence of functional groups associated with methane oxidation, while the relative contributions of aerobic and anaerobic methane oxidation will be evaluated based on the revised PHREEQC modelling results and their associated uncertainty.

Writing: The paper would benefit from a careful language and structure edit. Many key sentences rely on abstract nouns, passive constructions, and dense acronym use, which makes the main claims harder to follow than the underlying results. Consider using more concrete subjects and verbs, stating observations directly, and reserving hedging for the specific inference that is uncertain. Topic sentences should lead with the main finding, and the roles of PROFILE and PHREEQC should be signposted clearly. A thorough copyedit is also needed, as several grammatical errors currently hide scientific meaning rather than being merely cosmetic.

We thank the reviewer for this valuable comment. We agree that the readability of the manuscript can be substantially improved. In the revised manuscript, we will thoroughly revise the language and overall structure to improve clarity and readability. We will simplify sentence structure where appropriate, reduce the use of abstract nouns, passive constructions, and unnecessary acronyms, and present observations more directly while clearly distinguishing observations from interpretations. We will also revise topic sentences to better highlight the main findings of each section and improve the logical flow of the manuscript. As discussed in our responses to the modelling comments above, PROFILE will be removed from the revised manuscript, and the modelling section will therefore focus on PHREEQC and clearly describe its purpose and contribution to the study. Finally, the entire manuscript will undergo careful language editing and copyediting to correct grammatical errors and improve scientific communication.

Figures and tables: A clearer presentation of its quantitative results would be helpful. Please add tables summarizing site characteristics (a lot of key information is in text), PROFILE reaction zones and rates, and the PHREEQC kinetic parameters. In Fig. 2, the shared concentration scale makes low-abundance but important

species such as NO_2^- difficult to assess. Fig. 3b should report NMDS stress and formally test the claimed dispersion difference, while Fig. 5 should show variability between biological replicates. Fig. 4 would be more informative with model-fit uncertainty and the PROFILE-selected reaction zones clearly reported. Fig. 6 should include a sensitivity or uncertainty panel showing whether the strong pathway partition remains stable across plausible parameter values, rather than presenting only the selected simulation.

We thank the reviewer for these valuable suggestions. We agree that the presentation of the quantitative results can be improved. In the revised manuscript, we will reorganize the figures and tables to improve clarity and accessibility. We will provide summary tables for key site characteristics and PHREEQC kinetic parameters. Fig. 2 will be revised to improve the visualization of low-abundance species such as NO_2^-. For Fig. 3b, we will report the NMDS stress and statistically evaluate differences in community dispersion using PERMDISP, as described in our response to the microbiology comments above. Variability between biological replicates in Fig. 5 will also be presented more explicitly. Fig. 6 will be revised to incorporate the results of the uncertainty and sensitivity analysis and thereby show the robustness of the inferred pathway partitioning across the investigated parameter ranges. As discussed in our responses to the modelling comments above, PROFILE will be removed from the revised manuscript, therefore, the requested PROFILE reaction-zone and rate table and the corresponding PROFILE-related additions to the figures will no longer be applicable.

Technical Corrections

Spelling errors

- "is not well unterstood" should be "is not well understood." Done
- "and reation (monod kinetics)" should be "and reaction (Monod kinetics)." Monod should be capitalized throughout. Done
- "stabe isotope ratios" should be "stable isotope ratios." Done
- "can have fundamentally effects" should be "can have fundamental effects." Done

Grammar and missing words

- "there is no geochemical evidence of bacterial sulfate reduction from the pore water profiles at profile 1 suggests that..." is missing a clause boundary. Revise to "there is no geochemical evidence of bacterial sulfate reduction from the pore-water profiles at profile 1, which suggests that..." Done
- "sediment permeability may exert primary control hyporheic biogeochemical functioning" should be "sediment permeability may exert primary control over hyporheic biogeochemical functioning." Done
- "establishment of a denitrification zone, which in accordance with the classical redox sequence" should be "establishment of a denitrification zone, which is in accordance with the classical redox sequence." Done
- "where the greatest difference in redox are found" should be "where the greatest difference in redox is found." Done
- "considerable uncertainty can be assumed regarding their contribution" would be clearer as "considerable uncertainty remains regarding their contribution." Done
- "All data and code will be made available upon from the corresponding author upon request" should be "All data and code will be made available from the corresponding author upon request." Done

Correct precision

- "3290.64 and 3778.43 micromoles per liter" should be reported as approximately 3290 and 3780 micromoles per liter. Done
- "methane solubility of 1731.17 micromoles per liter" should be reported as approximately 1730 micromoles per liter. Done
- "a mean of minus 72.45 per mille" should be reported as minus 72.5 or minus 72 per mille. Done
- Rate constants such as "5.24 times 10 to the minus 6" and "6.77 times 10 to the minus 6" are reported with more precision than the inversion likely supports. Two significant figures would be more appropriate. Done

Terminology and consistency

- "micro-oxic," "suboxic," and "hypoxic" appear to be used somewhat interchangeably. Define the oxygen thresholds once and apply the terms consistently. Done
- "MOx" is introduced as methane oxidation but later appears to refer specifically to the aerobic methane-oxidation pathway. Use the abbreviation consistently. Done
- The formatting of delta 13 C methane is inconsistent. Standardize superscripts and subscripts throughout. Done
- "N-DAMO" and "nitrite-DAMO" are both used. Select one abbreviation and use it consistently. Done
- The primer sequences are written in three-letter groups. They should be presented as continuous nucleotide strings. Done

Citation and attribution

- The 515F and 806R primer pair is attributed only to Pichler and colleagues. The original primer design or modification papers should also be cited where appropriate. Done
- The Umezawa and colleagues 2025 reference appears incomplete and should include the full journal and DOI information. Done

Units and formatting: There is use of millimoles per liter, micromoles per liter, and millimoles per kilogram of water in different places. These units are all defensible, but their relationships should be stated clearly and used consistently when comparing text, figures, and model outputs.

Thank you for this helpful suggestion. We agree that the use of units should be more consistent throughout the manuscript. In the revised version, we will harmonize the units where possible and clearly state the relationships between the different units where comparisons are made, while consistently indicating the units used in the text, figures, tables, and model outputs to avoid ambiguity.

The approximation between millimoles per kilogram of water and millimoles per liter is stated once. Ensure that this conversion is clear wherever the two units are compared.

Thank you for pointing this out. We will ensure that the approximation between mmol kg^{-1} water and mmol L^{-1} is clearly stated wherever these units are compared in the revised manuscript.