

Reply on Reviewer Comment 1

General comments

Comment 1:

This is a nice and thorough study on metabolic rates in dynamic turbid river systems in a tectonic active area. The use of isotopic labelling of DIC and amino acids in combination with environmental parameter measured in field campaigns and characterisation of the microbial community composition is an impressive achievement. Although at some point it is questionable if all this information in one paper is contributing to the communication of the main message and findings (e.g. are alpha diversity, Shannon index beta diversity needed?). The storyline can be strengthened in the intro and discussion if more focussed hypothesis are formulated.

Reply 1:

Thank you for the reviewer's constructive feedback regarding the focus and storyline of the manuscript. While we agree that the metabolic rates are the primary focus of this study, we have chosen to retain the diversity data (Fig. 4a) as it provides critical evidence for the ecological resilience and metabolic stability of specific sites within this dynamic river system. To address the concern, we have revised the Introduction to provide a better cohesive narrative, specifically investigating whether extreme physical disturbances drive community shifts or if inherent resilience allows for stable carbon transformation. In the revised Discussion (section 4.6), we used these diversity indices to explain two distinct metabolic scenarios: i) seasonal sensitivity at high-sediment MLL01 and LY04 where significant reduction in alpha diversity during the wet season provides the ecological context for more variable metabolic rates; and ii) metabolic versatility at CWL01, where high baseline diversity and a complementary seasonal shift between Cyanobacteria and lithoautotrophs allow for stable carbon transformations despite high turbidity. The revised content (also in the Result, lines 345-348) makes the diversity indices from descriptive statistics into essential mechanistic context for the Beinan River's carbon cycle.

Comment 2:

The objective of this study was to get insights in the role of microbial CO₂ fluxes at catchment scale and disentangling the metabolic processes of autotrophic and heterotrophic CO₂ exchange in these understudied dynamic mountainous river systems in contrast to the more stable low-land catchments. The sampling strategy and major findings reported (seasonal shifts in metabolic wet-vs dry), although nice, do not seem to fully comply with my view of a study major strength to determine the effect of event-based flushes of groundwater and sediments on CO₂ fluxes. Furthermore, while Wang et al 2024 identified the effects of hot springs on the enrichment of waters with bicarbonate in the same sample locations in the tectonic active Beinan catchment this is not mentioned in this study. Is this not relevant in the total CO₂ flux or is it included?

Reply 2:

Thank you for the comment on the dynamic nature of the Beinan catchment and the role of groundwater and hot spring inputs. Regarding the sampling strategy, we agree that capturing discrete event-based flushes such as storm-triggered pulses of groundwater and sediment would be an ideal approach when studying CO₂ fluxes in mountainous systems. However, the logistical and safety constraints of performing *in situ* isotope labeling incubations during such extreme flow events is often a challenging consideration to acquire a representative sample. In the mountainous catchments in Taiwan, the high-energy wet season effectively represents a series of frequent event-based flushes that maintain the chronically high turbidity and sediment mobility we observed. Our bi-monthly sampling scheme was designed to establish a systematic seasonal baseline to evaluate metabolic stability across the hydrologic year, capturing the distinct transition between the dry (January - May) and wet (August) seasons of southeastern Taiwan. Notably, the August 2020 sampling coincided with high-turbidity conditions (Fig. S1), providing a representative data point analogous to 'event-like' conditions that can be directly contrasted with the lower-flow baseline periods. The sampling strategy has been further clarified in the revised manuscript (lines 120 - 124).

Regarding the potential influence of hot spring inputs, we have carefully evaluated our physicochemical dataset (Table S1) for signatures of hot spring inputs across all sites and seven field campaigns. We observed no localized anomalies in water temperature (T), suggesting that

geothermal inputs are primarily limited to the hot spring outcrops, not a prevalent distribution across the whole flow path. While some geochemical signatures (e.g., Cs ions) are sensitive to the detection of hot springs or subsurface high-temperature water-rock interactions in the region (Wang et al., 2024), water chemistry data revealed limited overall contribution of hot spring-sourced ions regardless of seasons. Furthermore, our molecular analyses did not detect a substantial presence of populations specifically originating from hot environments or groundwater. Taken together, geochemical and microbial community data indicate that hot spring or groundwater does not impose a high volumetric contribution to the river water, community composition, metabolism, or even CO₂ emission. Their contribution would be more significant at the intersection between hot fluid and river water. Nevertheless, the level of hot spring or groundwater contribution to river metabolism and community composition cannot be quantitatively constrained with the current experimental and sampling framework, and beyond the scope of the current investigation.

Comment 3:

What about redox situation in the streams influenced by tectonics? Is CH₄ exchange not relevant?"

Reply 3:

Thank you for the question regarding the redox status and the potential relevance of CH₄ exchange. Our unpublished *in situ* measurements conducted later (2023-2025) indicate that the Beinan River remains in a consistently oxidizing state, with oxidation-reduction potentials (ORP) staying positive across all sites (+18 to +238 mV). This is consistent with the fact that in such high-energy, turbulent mountainous streams, the steep topography facilitates rapid and continuous re-aeration, maintaining aerobic conditions throughout the water column. Methanogenesis typically requires strongly reducing conditions (negative ORP), which are absent in this system due to the lack of stagnant zones and frequent sediment scouring. Furthermore, the chronically low organic substrate availability in this catchment further limits the potential for microbial fermentation. Consequently, we consider CH₄ exchange to be a negligible component of the total carbon efflux compared to the dominant CO₂ evasion. Overall, the fundamental physical characteristics of the Beinan River - its steep gradient, high turbulence, and frequent sediment scouring - have enabled persistently aerobic conditions through time across the catchment.

Specific comments

Comment 4:

Abstract line 18 "several percent" please be more specific here. 107mol yr⁻¹ from microbial origin vs annual total emission across the catchment 2,6 .10⁹ ? (Line 295)."

Reply 4:

Thank you for the comment. We have updated the Abstract and the main text (lines 21 and 332-334) to provide the specific percentage as requested. Based on our scaled estimates, the total microbial net emission is 7.89 x 10⁷ mol yr⁻¹ (summed from individual tributaries in Table S5). When compared to the total annual CO₂ emission across the catchment calculated in Table S6 (average of 2.08 x 10⁹ mol yr⁻¹ across the five sampling campaigns), the microbial contribution represents approximately 3.8% of the total catchment-scale CO₂ evasion. We have replaced 'several percent' with 'approximately 3.8%' to be more precise.

Comment 5:

Line 38 landscape controlled or is it more topographically controlled?"

Reply 5:

Thank you for the comment. We have modified the sentence (line 42) to state that CO₂ flux is primarily topographically controlled in this study. In the upstream reaches of the Beinan River, the steep topographic relief and rugged channel morphology increase turbulence and gas transfer velocities, leading to the higher CO₂ evasion rates observed compared to the lower-gradient downstream reaches.

Comment 6:

Line 68 term “individual metabolisms” needs more clarification.

Reply 6:

Thank you for the comment. We have clarified the term “individual metabolism” in the revised manuscript (lines 74-77). It refers to the distinct metabolic pathways of carbon transformation quantified in this study, including photoautotrophy (light-dependent DIC fixation), chemoautotrophy (dark DIC fixation), and heterotrophy (mineralization of dissolved organic carbon).

Comment 7:

Lines 59-61 The information in this sentence is essential why this study in a rapid uplifting area is so different from the dominant body of literature in this field which is performed on the cratonic continent. While this relation between tectonics and torrential precipitation is a probably obvious for the authors this is not evident for everyone. It would help the storyline if this is relation between tectonics and dynamic, turbid high energy river systems is more explicit.

The expected deviations from the general benthic and hyporheic processes due to the turbid and dynamic river systems can be formulated more explicitly in hypothesis which will give their reader more guidelines for interpretation in the result section.

Reply 7:

Thank you for the comment. We have revised the Introduction (lines 72-74) to explicitly contrast our study system with the cratonic continental systems commonly addressed in the literature. We hypothesized that the intense geological and hydrological impacts of a rapid-uplift area create a physically unstable river environment. In such high-energy systems, the planktonic community constitutes a measurable and vital component of aquatic metabolism. Nevertheless, our incubation experiments only determine the metabolic activities and assemblages of planktonic communities. To constrain benthic and hyporheic processes and metabolic activities, oxygen sensors have to be deployed in the river channel. Once the activities of whole river metabolisms are deduced, the sum of metabolic activities in benthic and hyporheic processes can be constrained by subtracting the activities of planktonic metabolisms (like this study) from the whole activities. The deployment of sensors in turbid environments like those during high water time in Taiwan would be logistically challenging and beyond the scope of the current study.

Comment 8: Line 107-108, The selection of the 5 sample locations along the Beinan river and tributaries is not explained. Which criteria were used to determine these sample sites? Likewise no argumentation is provided for the selection of sample moments/ timing. As the dynamic nature of the Beinan river is a part of the research objective the regular bi-monthly sampling scheme is surprising. One would expect a focus on events (hot moments) and baseline moments.

Reply 8:

Thank you for the comment. We have revised the text (lines 119-124) to explicitly state that the five stations were chosen to represent individual sub-basins of the catchment and a topographic gradient that captures the transition from metamorphic headwater tributaries to the main stem. This sampling design allows us to observe how shifts in lithology and catchment characteristics influence microbial community structure and metabolic rates.

Regarding the sampling frequency, while we acknowledge the importance of “hot moments” (e.g., extreme storm events), our objective was to establish a systematic seasonal baseline to evaluate metabolic stability across the hydrologic year in such a dynamic system. The bi-monthly scheme was designed to capture the first-order seasonal transition between the dry (January - May) and wet (August) seasons of southeastern Taiwan. As discussed in reply 2, performing *in situ* isotope labeling incubations during peak storm discharge presents substantial logistical and safety challenges. Notably, the August 2020 sampling coincided with high-turbidity conditions, providing a valuable “event-like” data point to contrast with lower-flow baseline periods (Figure S1). While event-focused sampling would enable us to resolve the highly temporal variations in metabolic activity in response to extreme hydrological and geomorphic processes, our current sampling

design and interval offer the characterization of the metabolic activities representative for the investigated sub-basins over a yearly time scale. We are aware that the current dataset represents temporal snapshots of the planktonic metabolisms in a highly dynamic catchment. A long-term plan (probably at a decadal scale) of sampling and analysis would better resolve the variation in ecosystem function and activity and how hydrological and geological processes shape such variations.

Comment 9: Line 115. The use of cellulose membranes is not common practice and strongly discouraged in research on carbon dynamics due to the risks of contamination. Especially for DOC determination. The same is true for the use of polypropylene sample containers (risk of DOC contamination).

Reply 9:

Thank you for the comments on these technical details. We have clarified the materials for sampling and processing in the revised manuscript (lines 130-134) to correct these typos. The filters used were actually made of Supor® (Hydrophilic Polyethersulfone, PES), not cellulose. PES is a low-protein-binding material widely preferred in aquatic biogeochemistry for its minimal organic leaching and high flow rates. DOC samples were collected and stored in high-density polyethylene (HDPE) bottles, rather than polypropylene. To ensure data quality, all HDPE bottles were pre-cleaned (acid-washed and triple-rinsed with Milli-Q water). We also conducted procedural blanks by processing Milli-Q through the entire filtration and storage assembly. The resulting DOC concentrations in these blanks were consistently below the limit of detection, confirming that our materials did not introduce carbon contamination.

Comment 10:

Line 386: is this influenced or correlation based?

Reply 10:

Thank you for the comment. The statement regarding the relationship between DIC uptake rates and environmental variables (TSM, POC, $\delta^{13}\text{C}$ -POC, and ammonium) was based on Pearson correlation analysis ($p < 0.05$). To be more precise, we have rewritten the sentence (line 429) to state that these rates were significantly correlated with these parameters, rather than 'influenced' by them.

Comment 11:

Figure 3 needs a more elaborate figure caption.

Reply 11:

Thank you for the comment. We have significantly expanded the figure caption to clearly define the metabolic pathways represented in each panel: light/dark DIC uptake (autotrophy) and amino acid uptake/catabolism (heterotrophy). We have also added a note explaining that the error bars represent the standard deviation of triplicate measurements.