

Reply on
Report # 1
Anonymous referee # 3

As one of the original reviewers was unable to review the revised version of the manuscript, I am invited to serve as an additional reviewer for this revision. Overall, the authors have substantially addressed the concerns raised during the first round of review. The manuscript is now better structured, and the explanations regarding the role of planktonic metabolism in regulating CO₂ production and emissions are generally well supported and defensible. The findings from river catchments characterized by active tectonics and high soil erosion are valuable and will provide useful insights for future research on riverine carbon cycling.

I have only two minor comments for the authors to consider:

Comment 1:

The catchment-scale CO₂ flux estimates are highly dependent on the estimated surface area of the river network, as described in Section 2.5. Please clarify how the river surface area was quantified and provide an assessment of the uncertainty associated with these area estimates. In relation to this, the authors may need to propagate this uncertainty into the catchment-scale CO₂ flux estimates. For example, to what extent can CO₂ fluxes estimated from a single sampling point, such as the outlet of each sub-catchment, capture the spatial heterogeneity of CO₂ emissions across the entire catchment?

Reply 1:

Thank you for the comment. River surface area was calculated as 0.47% of the sub-catchment area (obtained from the Water Resource Agency hydrological atlas), following the global average reported by Raymond et al. (2013), as stated in Section 2.5. We did not quantify the exact river surface area using the image tools (e.g., satellite imaging) for several reasons: (1) The surface area of low-order reaches constitutes a significant proportion of the overall area across the catchment. However, their widths are commonly smaller than what most satellites can resolve (< 1 m) or potentially shaded by trees distributed along the bank; (2) The areas fluctuate greatly between events and with season in the mountainous region. A higher frequency of imaging with various tools would be needed to more accurately constrain the year-round area variations. Overall, it is technically challenging to fully recover the area variations for the surface areas of investigated tributaries over different temporal and spatial scales. Therefore, the average percentage of surface area based on a large dataset (Raymond et al., 2013) was adopted here, with a conservative $\pm 50\%$ uncertainty arbitrarily assigned.

To propagate this uncertainty into our catchment-scale estimates, we combined the $\pm 50\%$ river surface area uncertainty with the measured variability of metabolic or flux rates for

each site. For the planktonic CO₂ production, our revised calculation yielded a catchment-wise estimate of $7.99 \times 10^7 \text{ mol yr}^{-1}$ ($\pm 43\%$; range: $4.56 \times 10^7 - 1.14 \times 10^8 \text{ mol yr}^{-1}$). For the total river-air CO₂ evasion flux, our revised calculation yielded a catchment-wise estimate of $2.08 \times 10^9 \text{ mol yr}^{-1}$ ($\pm 54\%$; range: $9.57 \times 10^8 - 3.20 \times 10^9 \text{ mol yr}^{-1}$) (Table S6, S7).

Regarding extrapolation from a single sampling point, we acknowledge that one site per sub-catchment cannot resolve within-reach heterogeneity potentially contributed by the temporal and spatial variations in pCO₂. For example, groundwater is often characterized by a higher pCO₂, thereby leading to the potential enhancement of riverine pCO₂ if the groundwater discharge constitutes a high fraction of riverine discharge. Because the increase in pCO₂ can be gradually attenuated through the air-river exchange as the river flows downstream, the enhancement of riverine pCO₂ is likely to be observed. Our unpublished data measured across the catchment revealed a small variation in pCO₂ through time ($\pm 20\%$), a pattern consistent with the data collected for Alps streams (Horgby et al., 2019). As the flow along the mountainous region is rapid, it is also perceivable that planktonic communities and geochemical characteristics are well mixed along the flow path. Therefore, our sampling and incubation results may have captured the integrated average metabolic activity over the target sub-catchment. This limitation is discussed in Section 4.5.

Comment 2:

As shown in Fig. 1, agriculture appears to be an important land-use type within the study catchment. Could the elevated ammonium concentrations observed during the wet season (Line 463) reflect agricultural inputs or runoff? The authors may need to discuss this potential influence and its implications for interpreting seasonal nitrogen dynamics and CO₂ emissions.

Reply 2:

Thank you for the comment. We agree that agricultural land use is more extensive in the lower catchment (Fig. 1b) and represents a plausible additional source of ammonium during the wet season, as described in Section 4.1. We also note that the porewater accumulation and flush discussed in this section could be a plausible cause regardless of whether the ammonium originates from the degradation of natural organic matter or agricultural inputs. Identifying the exact source contribution would require a diagnostic marker (e.g., isotope composition, or close monitoring of ammonium concentration against discharge) currently not available. We further note that this uncertainty does not affect our carbon-based metabolic rate interpretations or catchment-scale CO₂ flux estimates.

Reference

Horgby, Å., Segatto, P. L., Bertuzzo, E., Lauerwald, R., Lehner, B., Ulseth, A. J., Vennemann, T. W., and Battin, T. J.: Unexpected large evasion fluxes of carbon dioxide from turbulent streams draining the world's mountains, *Nat. Commun.*, 10, 4888, <https://doi.org/10.1038/s41467-019-12905-z>, 2019.

Raymond, P. A., Hartmann, J., Lauerwald, R., Sobek, S., McDonald, C., Hoover, M., Butman, D., Striegl, R., Mayorga, E., Humborg, C., Kortelainen, P., Dürr, H., Meybeck, M., Ciais, P., and Guth, P.: Global carbon dioxide emissions from inland waters, *Nature*, 503, 355–359, <https://doi.org/10.1038/nature12760>, 2013.

Reply on
Report # 2
Anonymous referee # 4

GENERAL COMMENTS

I appreciate the time and effort that the authors have invested to address previous comments. Most major concerns have been addressed. However, further details can still be added to certain parts of the methods section. Moreover, the discussion section is occasionally descriptive and not sufficiently interpretative. The authors should ensure that it is entirely devoted to offering explanations for their data.

Reply: Thank you for the comments. We have supplemented details of certain analytical methods. We have gone through the discussion section and provided additional explanation and justification about the interpretation and limitations of the current data sets.

SPECIFIC COMMENTS

Comment 1:

Lines 14-15: Functional expression of what? Microbial communities?

Reply 1:

Thank you for the comment. We have revised the sentence to make it explicitly clearer.

Comment 2:

Line 174: Please mention in the main text that this 'near-ambient temperature' indeed varied with season. What exactly were the temperatures? It would be helpful to explicitly state them or at least direct readers to the values recorded in Table S1.

Reply 2:

Thank you for the comment. We have clarified in the Methods that "near-ambient temperature" tracked seasonal air temperature, with an average daily range of 19.3 to 27.2 °C across the sampling campaigns (CODiS, CWA Observation Data Inquiry Service, Central Weather Administration), using records from four nearby weather stations: Taitung (467660), Luye (C0S710), Chihshang (C0S740), and Xiama (C0S660).

Comment 3:

The authors have now clarified that aquatic metabolisms involving pre-existing POC were not measured. However, as they suggest, these mountainous systems are unique because of their turbidity during high-flow periods. It therefore seems a bit counterintuitive that the biological role of suspended particulate matter was not considered. Since the authors only measured the transformations of DOC, could they elaborate somewhere in the methods section (e.g. in the paragraph preceding Figure 2) how DOC is particularly

relevant to these mountainous systems? Perhaps emphasise that DOC, to a certain extent, originates from the breakdown of suspended POC?

Reply 3:

Thank you for the comment. River metabolisms represent an amalgamation of complex metabolic processes in river systems. Substrate availability and microbial communities inhabiting different compartments all contribute to the resulting metabolic patterns and rates. To assess the role of POC in river metabolisms, our previous study using Raman spectroscopy combined with isotopic evidence demonstrates that suspended POC is primarily composed of rock-bound organic carbon (OC), rather than soil OC (Lien et al., 2025). Since the river transport is rapid and rock-bound OC is recalcitrant, the degradation of suspended POC along river transit would be limited (Lien et al., 2025). Similar observations were also obtained from Hilton et al. (2008), from which the radiocarbon contents of suspended POC were generally low during most periods. Instead of substrate exploitation, our analysis revealed that leucine uptake rates were correlated with particulate characteristics (section 4.4). The pattern suggests that leucine uptake is linked to the size of the population attached to particulate organic matter (Fig. S4). With a larger influx of TSM in the wet season, the size of the attached population could have been correspondingly enhanced, providing a larger population of microorganisms with functional preferences for leucine uptake. In this regard, POC may serve as the source of target metabolizers for different tributaries and/or under different physicochemical conditions. As our catchment system is characterized by the limited development of soil, DOC and nutrients leached from soils and exported into the river are scarce. Therefore, we chose DIC and amino acids as our target substrates to address how fast autotrophs and heterotrophs can metabolize the dissolved substrates readily for utilization and whether river metabolism impacts the catchment carbon budget in such DOC-lean mountainous catchments. We have incorporated the discussion and statement into the manuscript.

Comment 4:

Line 187: What water samples were measured? Those collected in the field, as well as those extracted at the beginning and the end of each incubation trial? It is not currently clear in the text.

Reply 4:

Thank you for the comment. Anions, major cations, orthophosphate, ammonium, nitrite/nitrate, and Chl a were measured only for river water samples, as already specified in Section 2.2. DIC and POC were measured in both field and incubation samples. We have revised the text accordingly for clarification.

Comment 5:

Line 210: Were all three rates calculated separately for light and dark treatments respectively? Please specify in the text.

Reply 5:

Thank you for the comment. The DIC uptake rates ($\rho_{\text{DIC_uptake}}$) for light and dark conditions were calculated separately. The uptake and catabolic rates for the amino acid incubation ($\rho_{\text{AA_uptake}}$ and $\rho_{\text{AA_catabolic}}$) were conducted under dark conditions only. The details of the incubation setup were described in the previous section (Section 2.2).

Comment 6:

Please report the statistical results for parameters that were significantly higher/lower (e.g. lines 306-309, 312, 315, 316, 347, 348, 351, 352, 356). Moreover, please report test statistics alongside p values whenever statistical test results are provided (e.g. lines 325, 327, 349).

Reply 6:

Thank you for the comment. We have added a new Supplementary Table (Table S2) reporting the full linear mixed-effects model results (estimate, SE, t , df, p) for all physicochemical parameters reported in this paragraph. The p -values are indicated in the revised manuscript.

For the Kruskal-Wallis test results referenced at lines 325, 327, and 349, we have added the corresponding test statistics alongside the p -values throughout the Results section. Specifically, within-site seasonal variation in DIC uptake rates (light conditions) is now reported with individual H-values for each site. We also identified and corrected a discrepancy in the previously reported Kruskal-Wallis results for Figure 3 panels (a) and (d), which are now $H = 25.07$, $p < 0.0001$ (panel a) and $H = 15.47$, $p = 0.004$ (panel d; previously reported as non-significant). The correction does not affect the overall conclusions regarding net heterotrophy or the contribution of planktonic metabolism to catchment CO_2 evasion, nor the seasonal variation or substrate-preference discussions elsewhere in the manuscript. The spatial-pattern discussion in Sections 4.2 and 4.4 has been updated accordingly to reflect the corrected statistics.

Comment 7:

Lines 323, 346, 350: Please use the symbols defined earlier for the various rates.

Reply 7:

Thank you for the comment. The sentences have been revised with the corresponding symbols.

Comment 8:

Lines 358-364: Please specify whether the correlations were positive or negative.

Reply 8:

Thank you for the comment. The relevant sentences have been revised to explicitly state the trend (positive or negative) of each correlation.

Comment 9:

Lines 471-472: The authors mention here that nutrients do not seem to be an important factor controlling phototrophy, but this contradicts what is stated later in line 503.

Reply 9:

Thank you for the comment. The statement in Section 4.1 was intended to describe MLL01 and LY04 specifically, following from the preceding sentence discussing Chl a at these two sites, where nutrient concentrations do not vary considerably and do not appear to control phototrophic activity. We have revised the sentence to explicitly describe this scope.

Comment 10:

Line 491: The authors could summarise the limiting factors for the different sites in a more explicit way – light for first two sites and nutrient for the other three sites.

Reply 10:

Thank you for the comment. We have added a summary sentence at the end of this section explicitly stating that phototrophic rates were primarily light-limited at MLL01 and LY04, and nutrient-limited at CWL01, DL, and BNE.

Comment 11:

Section 4.3: This is a very long paragraph! Please consider breaking it down into shorter paragraphs for greater clarity.

Reply 11:

Thank you for the comment. We have split this paragraph into three for better clarity.

Comment 12:

Lines 550-551: This statement contradicts the information given in Figure 3c, which shows higher leucine uptake rates in the downstream sites.

After all, did the five sampling sites exhibit significant differences in rates of autotrophy and heterotrophy? What would be the reasons for the (lack of) spatial differences? In Sections 4.3 and 4.4, there are attempts to explain seasonal differences, but answers to the above questions about spatial trends are still not obvious.

Reply 12:

Thank you for the comment. The statement regarding leucine uptake favoring upstream is partly correct. Among sites, BNE and LY04 were characterized by high leucine uptake rates. While BNE is located downstream of all the other sites, LY04 is the major tributary of BNE and is located not too far from BNE. Therefore, the TSM of LY04 could be easily propagated to BNE (499 vs. 106 mg L⁻¹ for the average wet season in Table 2). This is

why the statistical correlation is apparently dominated by the high-TSM sites but is not completely consistent with the upstream-downstream distribution (Fig. 3c). We have revised this sentence to describe the correlation with TSM/POC characteristics.

Regarding spatial differences, autotrophic DIC uptake rates (light conditions) differed significantly among sites (Kruskal-Wallis, $H = 25.07$, $p < 0.0001$; reported in Results and Fig. 3 caption), with BNE significantly higher than MLL01, CWL01, and LY04 (Dunn's post-hoc, $p < 0.05$). This spatial pattern is discussed in Section 4.2, which links the site groupings to light limitation by suspended sediment (at MLL01 and LY04) versus nutrient availability (at the other sites, particularly BNE).

For heterotrophic metabolism, no significant difference was detected among sites for uptake rate (Kruskal-Wallis, $H = 5.68$, $p = 0.224$), whereas catabolic rates differed significantly among sites ($H = 15.47$, $p = 0.004$), with BNE higher than DL and LY04. We have added these statistics to Section 4.4, along with a brief discussion noting that this divergence between assimilation and catabolism is consistent with the accumulation of nutrients and degradable organic matter toward the estuary (Section 4.1), even though amino acid uptake capacity itself appears broadly uniform across the catchment.

Comment 13:

Line 631: Are there previous studies that only considered planktonic rates as well? How do results of this study compare to those?

Reply 13:

Thank you for your comment. We are aware of limited previous work that isolates planktonic (water-column) metabolism from benthic contribution. We have added a comparison to Rovelli et al. (2017), who used bottle incubations to measure water-column metabolism separately from benthic components in UK headwater streams. Their water-column rates (peak $2.5 - 3.5 \text{ g C m}^{-2} \text{ d}^{-1}$) are comparable to our planktonic heterotrophic fluxes ($0.055 - 1.85 \text{ g C m}^{-2} \text{ d}^{-1}$), despite their water column contributing a substantially higher fraction (25%) of total ecosystem metabolism than our planktonic contribution to total CO_2 evasion ($< 5\%$). The different fractional contribution may arise from differences in landscape and hydrology gradients, pending further systematic verification. We have clarified in the manuscript that this difference in fractional contribution reflects the dominance of weathering-derived CO_2 in our system's evasion budget, rather than an anomalously low planktonic metabolic rate.

Comment 14:

Lines 635-654: Current wordings of this paragraph make planktonic metabolisms sound insignificant. Since planktonic metabolism is the focus of this paper, the authors should instead highlight that a 4% contribution to carbon evasion is not negligible. Some of the cited studies found that NEP contributes to approximately 10% of total carbon evasion. If

planktonic metabolism (one part of NEP only) alone already contributes 4%, it certainly cannot be ignored.

Reply 14:

Thank you for the comment. We agree that a planktonic-only contribution of approximately 4%, compared to the 7-50% of NEP contributions reported in previous studies, indicates that planktonic metabolism is not a negligible component of catchment carbon cycling. This point is already addressed in the preceding paragraph, where we note that because those NEP estimates integrate planktonic, benthic, and hyporheic contributions collectively rather than separating them, our planktonic-only rates suggest that the planktonic compartment can be a significant fraction of total stream respiration. We have further strengthened this point by comparing our rates with Rovelli et al. (2017), who measured water-column-only metabolism using bottle incubations in headwater streams (see previous reply); our planktonic rates fall within the range they reported, reinforcing that the low fractional contribution of planktonic metabolism to total CO₂ evasion in our system reflects the dominance of weathering-derived CO₂ in this catchment, rather than an intrinsically low planktonic metabolic rate.

Comment 15:

Section 4.6 has been improved, as there is now an attempt to link specific taxa to the biogeochemical processes observed. However, it is still very descriptive and reads more like a results section. There is little explanation for the spatial and seasonal dynamics observed. In other words, why were microbial communities different between different sites and between different seasons? This is the question that needs to be addressed in a discussion section.

Reply 15:

Thank you for the comment. We have revised Section 4.6 to address why microbial community composition varied spatially and seasonally across the catchment. Specifically, we propose that the upstream sites are underlain by metamorphic rocks inherited with reduced iron and sulfur species readily available for lithoautotrophy. For comparison, the most downstream site (BNE) integrates organic matter and nutrients accumulated from the upstream catchment, potentially favoring heterotrophic dominance. This longitudinal shift from geochemically-driven to organic-matter-driven metabolism is discussed in the context of established river network concepts (e.g., the River Continuum Concept; Vannote et al., 1980). We also found that hydrological variability shaped community composition through several distinct, site-dependent processes rather than a single uniform mechanism: at sites with clear seasonal structuring (MLL01, CWL01, LY04), dominant lithoautotrophic taxa shifted between dry and wet periods, a pattern consistent with reorganization of redox substrate availability; at DL, community composition instead tracked dissolved organic carbon concentration rather than season; at BNE, communities were dominated by heterotrophs and highly variable regardless of

season. As we did not directly measure redox conditions, light attenuation, or groundwater contributions, the geochemical explanation for the upstream taxa is presented as an inference consistent with the observed community composition, rather than an independent validation. Please also note that our data are DNA-based, a measure of cell abundance rather than cell activity. Even though we have observed specific taxa that can be linked or inferred to specific metabolisms, their abundances are not necessarily reflected in their ecological significance. That is why our interpretations remain limited to what the data can support. These revisions have been incorporated into Section 4.6.

Reference

Hilton, R. G., Galy, A., Hovius, N., Chen, M.-C., Horng, M.-J., and Chen, H.: Tropical-cyclone-driven erosion of the terrestrial biosphere from mountains, *Nat. Geosci.*, 1, 759–762, <https://doi.org/10.1038/ngeo333>, 2008.

Lien, W.-Y., Chen, C.-T., Lee, Y.-H., Su, C.-C., Wang, P.-L., and Lin, L.-H.: Two-stage oxidation of petrogenic organic carbon in a rapidly exhuming small mountainous catchment, *Commun. Earth Environ.*, 6, 45, <https://doi.org/10.1038/s43247-025-02015-8>, 2025.

Rovelli, L., Attard, K. M., Binley, A., Heppell, C. M., Stahl, H., Trimmer, M., and Glud, R. N.: Reach-scale river metabolism across contrasting sub-catchment geologies: Effect of light and hydrology, *Limnology & Oceanography*, 62, <https://doi.org/10.1002/lno.10619>, 2017.

Vannote, R. L., Minshall, G. W., Cummins, K. W., Sedell, J. R., Cushing, C. E.: The River Continuum Concept, *Can. J. Fish. Aquat. Sci.*, 37, 130-137, <http://doi.org/10.1139/f80-017>, 1980.