Reply to Editor comment

GENERAL COMMENTS

This manuscript documents a field study conducted along the Beinan River in eastern Taiwan. The authors attempted to provide a novel account of microbial processes within the water column and consider how these processes contribute to catchment-wide carbon cycling and emission patterns. In general, ideas are presented clearly throughout the manuscript. However, a more comprehensive consideration of all the relevant biogeochemical processes (e.g. benthic metabolism and biodegradation of suspended particulate organic carbon) should be included. More details are required in certain parts of the text, particularly in the methodology section. Some comparisons in the results section are currently qualitative, and appropriate statistical tests should be reported to provide stronger quantitative evidence. Moreover, spatial (longitudinal) variations, or their lack, should be further addressed in the discussion. These concerns need to be addressed before I can fully support publication.

Reply to general comment:

We thank the editor for the constructive overview and for guiding both the general and specific issues. The four broad concerns have been addressed through substantial revisions across the manuscript.

i) Comprehensive consideration of biogeochemical processes. The Introduction and Discussion have been revised to make planktonic-specific framing explicit, with benthic and hyporheic processes now acknowledged as important contributors to whole-stream metabolism in many upland systems. A literature-based comparison of our planktonic metabolic rates with published respiration rates from morphologically comparable upland streams has been added in Section 4.5 (Replies 4, 26, 31, 33). The Conclusions and several discussion passages have been revised accordingly (Replies 31, 32, 34, 35, 39). POC biodegradation as a parallel water-column heterotrophic pathway is now explicitly acknowledged (Reply 14).

ii) Methodology details. Section 2 has been expanded with detailed descriptions of the incubation experiments, including the bottle materials, substrate concentrations, light and temperature conditions, incubation duration, and rationale for substrate selection (Replies 8-13).

iii) Statistical tests for previously qualitative comparisons. Quantitative tests have been added throughout: linear mixed-effects models for seasonal differences in environmental parameters (Reply 20), PERMANOVA for community composition (Reply 22), envfit for environmental drivers of community structure (Reply 23), Kruskal-Wallis for alpha diversity (Reply 17), and Kruskal-Wallis with Dunn's post-hoc tests for among-site differences in metabolic rates (Reply 21). All test statistics are reported in the Results, figure captions, and revised Methods.

iv) Spatial (longitudinal) variations. Spatial trends have been added or strengthened in both the Results and the Discussion. The downstream gradient in ammonium concentrations is discussed in Section 4.1 (Reply 24); site-specific patterns of lithoautotrophy and heterotrophy are addressed in Sections 4.3 and 4.4.

Together with the specific revisions detailed in our point-by-point responses below, these changes substantially improve the comprehensiveness, methodological transparency, statistical rigor, and spatial discussion of the manuscript.

SPECIFIC COMMENTS

Comment 1:
Line 1: The study only considers microbial activities in the water column. The title should specify 'roles of pelagic (or planktonic) microbes' or 'roles of microbes in the water column' instead of simply stating 'microbial role'.
Reply 1:
Thank you for the comment. We have revised the title to "Roles of planktonic metabolism in CO_2 fluxes along the river-estuary continuum in a rapidly uplifting catchment of eastern Taiwan" to accurately reflect the scope of the study.

Comment 2:
Line 18: Give a precise percentage instead of simply saying 'several percent'.
Reply 2:
Thank you for the comment. This concern was also raised by Reviewer 1 (comment 4), and we have addressed it by replacing the vague "several percent" with the specific value "approximately 3.8%" in the Abstract. This value was derived from the scaled estimates in which the total planktonic microbial net emission (7.89×10^7 mol yr^{-1}, summed from individual tributaries in Table S5) was compared against the total catchment-scale CO_2 evasion (average of 2.08×10^9 mol yr^{-1} across the five sampling campaigns; Table S6). The corresponding calculation and its interpretation are now described in the Results section.

Comment 3:
Line 20: Microbes that metabolise sulphur or nitrogen can also be heterotrophs (e.g. sulphate reduction or denitrification). Please specify which sulphur or nitrogen metabolic pathways are being referred to here.
Reply 3:
Thank you for the comment. In our system, the taxa driving the wet season community shift at the mountainous sites are chemolithoautotrophic sulfur- and ammonia/nitrite-oxidizers that couple the oxidation of reduced sulfur and nitrogen compounds (sulfide/thiosulfate, ammonium, nitrite) to most likely oxygen consumption with CO_2 fixation via the Calvin-Benson-Bassham cycle. Specifically, the dominant wet-season members identified in our 16S rRNA gene dataset include sulfur-oxidizing *Thiobacillus*, *Sulfuricurvum*, *Sulfurovum*, *Sulfurifustis*, and *Thiothrix*, together with ammonia/nitrite-oxidizing *Nitrosomonas*, *Nitrosopumilus,* and *Nitrospira*. Canonical heterotrophic sulfate reducers or denitrifiers were not observed as significant community members. This specific interpretation is already elaborated in the Discussion (section 4.3) and stated in the Conclusion, where these taxa are explicitly classified as lithoautotrophs. The statement has been revised in the Abstract and in Conclusion.

Comment 4:
Lines 66 to 68: This sentence (not substantiated by any references) cannot justify why planktonic processes would dominate in this system. Excluding benthic metabolism constitutes a striking omission. Many previous studies have found that, at least in upland streams, benthic processes contribute a much greater proportion to whole-stream metabolism than planktonic processes (e.g. https://doi.org/10.1029/2024WR039373). As presented later in Table 2, the upstream sites had TSM around 10 mg/L, particularly in the dry season. The water was therefore not very turbid, and sunlight should be able to reach the benthic algae growing on coarse stream bed sediments. Even

if no benthic data were collected, the authors should compare their planktonic results to known rates of benthic activities from nearby or similar streams.

Reply 4:

Thank you for the comment. Our intended meaning in lines 66-68 was that, within the methodological framework of this study (closed ^{13}C-labeling incubations of river water), the planktonic compartment is the target component directly quantified for its activity — not that planktonic processes dominate the total aquatic metabolic flux in this catchment. The experiment was designed specifically to isolate water-column metabolic activity, not to resolve benthic or hyporheic processes. We acknowledge, however, that the original wording "…the primary measurable component of aquatic metabolism" is ambiguous and can be recognized as a claim about relative magnitude across compartments, which was not our intent. We also acknowledge that the dry-season upstream TSM values (~10 mg L^{-1}) are low enough that light can reach the stream bed, so benthic primary production is not physically precluded in all of our sampling conditions, and that benthic metabolism is in principle measurable with other established approaches (e.g., open-channel O_2 mass balance, benthic chambers, sediment-core incubations) that lie outside the experimental scope of this study.

We have revised the manuscript accordingly. The revised statement in Introduction explicitly acknowledges that benthic and hyporheic compartments are widely recognized as important contributors to whole-stream metabolism in many upland systems, reframes the planktonic compartment as the component methodologically accessible to closed-bottle isotope labeling, and restates the hypothesis as a testable proposition about seasonally variable planktonic activity rather than an assertion of planktonic dominance.

Further discussion has been added in section 4.5 that places our planktonic rates in context with rates reported for morphologically comparable upland streams. For comparison, we relied on studies reporting direct measurements of gross primary production (GPP) and ecosystem respiration (ER). Our measured autotrophic DIC uptake rates (0.03-1.98 mg C m^{-3} h^{-1}) correspond to approximately 4×10^{-4} to 2×10^{-2} g C m^{-2} d^{-1} for a representative mean depth of ~0.5 m, which is one to two orders of magnitude lower than benthic gross primary production typically reported for clear upland streams (~0.1 to several g C m^{-2} d^{-1}; Hall et al., 2015; Bernhardt et al., 2022). Planktonic autotrophy in the Beinan River is therefore clearly a minor contributor relative to benthic primary production in comparable clear upland systems. Our measured planktonic heterotrophic catabolic rates (0.055-1.85 g C m^{-2} d^{-1} on an areal basis), in contrast, fall within the typical range of total ecosystem respiration reported for upland streams (~0.5 to several g C m^{-2} d^{-1}; Battin et al., 2008; Hall et al., 2015; Bernhardt et al., 2022). Because ecosystem respiration in those studies encompasses planktonic, benthic, and hyporheic contributions rather than separating them, our planktonic-only rates suggest that the planktonic compartment can be a substantial fraction of total stream respiration. More detailed measurements are warranted to validate whether different compartments of the Beinan river systems contribute to the net carbon export in a pattern resembling other similar catchments.

Comment 5:

Section 2.1: Are there human activities in the catchment? What different land use or land cover types are there? This information can be included in Figure 1.

Reply 5:

Thank you for the comment. We have revised the content in Section 2.1 and updated Figure 1 to include a land-use/land-cover panel (Fig. 1b), derived from the National Land Use Investigation dataset (National Land Surveying and Mapping Center, Ministry of the Interior, Taiwan). The Beinan catchment is located in eastern Taiwan and, in contrast to the more densely urbanized and cultivated catchments of western Taiwan, the landscape is dominated by steep, forested mountain terrain in its upper and middle reaches. Agricultural and populated areas are concentrated in a narrow band along the lower Longitudinal Valley and the coastal alluvial plain near the estuary.

Comment 6:

Lines 107 to 108: What are the physical characteristics of these five sites (e.g. gradients, channel widths and depths, sedimentary characteristics, flow rates)? To what extent was the most downstream site influenced by seawater? How and why were these sites selected? Can the authors provide field photos of the study sites? These can help readers understand whether benthic processes are really (un)important.

Reply 6:

Thank you for the comment. The rationale for selecting the five sampling sites has been added in response to Reviewer 1 (Reviewer 1's Comment 8; revised text at lines 124-139). Comprehensive physical and chemical characteristics for each campaign is tabulated in Table S1. In response to the editor's request for a fuller physical characterization, we have additionally expanded Section 2.1 with a descriptive paragraph on channel characteristics, substrate, and hydraulic setting. We also added *in situ* salinity and surface flow velocity as new columns in Table S1. Finally, we included field photographs of all five sampling sites as a new supplementary figure (Fig. S2).

The five sites were selected to facilitate the retrieval of microbial characteristics of major tributaries that span over a morphological transition with different lithological units and types of land use. MLL01 is located in the upper tributary headwaters of the Beinan catchment and is characterized by a steep, boulder-dominated channel within a forested valley where sediments are generated primarily from the slate belt. CWL01 and DL are mid-catchment sites on the upper main stem and an adjacent tributary, respectively, both with coarse-grained beds, and extensive exposure of gravel bars during low flow. Both sites received sediments composed of primarily schist and slate with minor contributions of marble and metasandstone. LY04 is on the lower tributary, where the channel broadens into a wider, cobble-bed reach flanked by agricultural terraces. BNE is at the river mouth of the Beinan River.

Regarding the seawater influence, *in situ* salinity at BNE ranged from 0.29-0.59 ppt across all seven campaigns (updated Table S1), a range only marginally higher than the upstream freshwater sites (0.22-0.52 ppt) and well below brackish-water levels. Consistent with this, the major-ion chemistry in Table S1 shows Cl^- at BNE of 0.22-0.28 mM and Na^+ of 0.65-0.77 mM, a range several orders of magnitude below ambient seawater values (~550 mM Cl^-, ~470 mM Na^+), and comparable to or lower than values observed at upstream freshwater sites. Together these two independent lines of evidence indicate that BNE was dominated by riverine freshwater in all sampled campaigns and that seawater intrusion was negligible. We therefore interpret results from BNE as representing the freshwater end of the river-estuary continuum under the sampled conditions, rather than a fully mixed estuarine environment. Panel E of Figure S2 shows BNE and its engineered wave-dissipating tetrapod structures immediately inshore of the river mouth. Revised sentences have been added to Section 2.1.

Comment 7:

Line 109: Please provide quantitative river discharge information for each sampling month.

Reply 7:

Thank you for the comment. We have compiled quantitative river discharge data from the Water Resources Agency (WRA) Hydrological Yearbook for the 2020-2021 sampling period and added these as a new column in Table S1.

Of the WRA gauging stations operating in the Beinan catchment during the sampling period, three correspond to our sampling sites and provide daily mean discharge on each sampling date, including station 2200H011 (Taitung Bridge) on the main stem at BNE; station 2200H021 (Dalun) located in the lower reach of Dalun River immediately above its confluence with Xinwulu River and

in close proximity to our DL sampling site; and station 2200H029 (Luming Bridge) on Luye River at LY04. The full station-by-campaign discharge values are now tabulated in Table S1.
For the two other sites (MLL01 and CWL01), no WRA gauging stations are deployed in their immediate sub-basins, and direct discharge values are therefore not available. We have added a sentence acknowledging this limitation, and directed readers to the monthly precipitation panels in Figure S1 at the hydrological context for these two sites.

Comment 8:
Lines 116 to 117: Since the procedures for measuring these chemicals are only described later in the text, it is not necessary to list all of them out here.
Reply 8:
Thank you for the comment. The list at lines 116-117 is part of the sampling procedure description rather than analytical methods to indicate the dissolved-phase parameters obtained from the same 0.22 μm PES filtration aliquot. We have therefore retained the category-level list, but we agree that the parenthetical detail of nutrient species is redundant and have been removed.

Comment 9:
Line 131: Why were these two amino acids selected in particular?
Reply 9:
Thank you for the comment. The rationale was addressed in our response to Reviewer 2 (Comment 8) and discussed in Section 4.4. In brief, glycine and leucine are dissolved free amino acids (DFAA), the most bioavailable fraction of dissolved organic matter (Kirchman, 2001). The two were selected to capture contrasting heterotrophic strategies. Leucine is the standard proxy for bacterial protein synthesis (Kirchman et al., 1985) and reflects predominantly anabolic activity. Glycine, the simplest amino acid (C:N = 2:1), is processed through rapid catabolic pathways and represents the labile, fast-cycling DFAA pool. Together, these two substrates bracket the range of heterotrophic strategies within the riverine microbial community.

Comment 10:
Lines 136 to 137: Why did the 13C-enriched DIC need to have lower concentrations than the natural environment?
Reply 10:
Thank you for the question. The ^{13}C-DIC tracer was added at a concentration substantially below the ambient DIC pool to avoid the substrate stimulation under most incubation conditions, so the measured ^{13}C uptake rates would resemble more closely the rates manifested by natural populations. The wording has been revised to express this quantitatively as a ~5% enrichment of the ambient DIC pool.

Comment 11:
Line 140: More information about the incubation experiments needs to be given. Where were the experiments conducted? In the field (as suggested by the title of Section 2.2), or in the lab? How many replicates were there for each site in each sampling month? What volume of water was incubated? Was there any headspace in the incubation container?
Reply 11:
Thank you for the comment. We have expanded Section 2.2 to provide the experimental details, and added a conceptual schematic of the setup as Figure 2.

i) Location. Water collection, ^{13}C-labelled substrate addition, and the incubation itself were carried out at the field accommodation where the team was based during each campaign. Filtration was performed immediately at the end of each incubation.
ii) Replication. Triplicate incubations (n=3) were prepared for the light ^{13}C-DIC autotrophy and for both amino acid heterotrophy treatments (^{13}C-glycine and ^{13}C-leucine). The dark ^{13}C-DIC condition was conducted as a single incubation (n=1) per site per sampling campaign. Each site therefore received 10 simultaneous bottles: 3 light and 1 dark for ^{13}C-DIC autotrophy, 3 dark for ^{13}C-glycine heterotrophy, and 3 dark bottles for ^{13}C-leucine heterotrophy. A T0 sample (1L of substrate-amended water filtered immediately after substrate addition and before incubation) was also processed for each incubation as the initial reference point.
iii) Volume. Six liters of river water were collected per substrate type. For ^{13}C-DIC autotrophy, the 6 L bag was split between the 3 light incubation bottles and the 1 dark incubation bottle. For incubations with two heterotrophy substrates (^{13}C-glycine and ^{13}C-leucine), incubations of each substrate were performed on individual 6 L bags. The total volume of water collected for incubations added up to 18 L (three 6 L bags) per site per campaign. After substrate addition (6 mL of 50 mM stock per 6 L bag, final concentration 50 μM), the amended water was distributed into 1L HDPE incubation bottles (transparent for the light ^{13}C-DIC bottles, and opaque for the dark ^{13}C-DIC bottle and the heterotrophy treatments).
iv) Headspace. All bottles were completely filled to eliminate headspace and prevent gas exchange during the incubation.

Comment 12:
Line 141: Did all set-ups receive light? Figure 2 suggests that some samples were incubated in darkness, but this information was not stated in the main text.

Reply 12:
Thank you for your question. Not all setups were subject to light. For the ^{13}C-DIC autotrophy incubation, three transparent (light) HDPE bottles and one opaque (dark) HDPE bottle were prepared per site per campaign. The light bottles were incubated under constant artificial illumination at ~18,000 lux, monitored by a HOBO Pendant MX logger, while the dark bottle was incubated alongside in the dark. For the ^{13}C-amino-acid heterotrophy incubations (glycine and leucine), three opaque HDPE bottles were prepared per substrate and incubated under dark condition only.

Comment 13:
Line 142: Why were all samples incubated under room temperature instead of field temperature? Temperature heavily influences metabolism. How could in situ rates be approximated if the natural water temperature was not used? Moreover, if temperature was kept constant, how could seasonal variation in metabolic rates be revealed?

Reply 13:
Thank you for the question. We agree that temperature influences metabolism and that lab-temperature incubations cannot replicate *in situ* rates. The original phrase "room temperature" at line 142 was intended to refer to the ambient temperature of the field site rather than to any lab-controlled value, but we recognize that this could be recognized either way. Therefore, the text has been revised.

Specifically, autotrophy bottles were placed in a flowing tap-water bath at the field accommodation for the duration of the incubation, both to dissipate heat from the lighting unit and to keep bottle temperature close to ambient surface water temperature; local tap-water temperature was similar to ambient surface water temperature, within a few degrees. Heterotrophy bottles were incubated under the same condition. We acknowledge that the incubated temperatures were neither constant nor the same as field value even though the difference may be small. Therefore, the target metabolic activity can be impacted at various degrees, depending on whether the target metabolisms or community members are sensitive to the incubated temperature range. It is also likely that the small difference in temperature described above is far smaller than the seasonal range across campaigns (ambient water temperature varied from 12.3 ˚C in January to 30.4 ˚C in August; Table S1). Therefore, we consider that our incubation setting still approximate the field condition to the greatest degree we can achieve.

Comment 14:
Figure 2: Did the authors consider the biodegradation of suspended POC as well? If not, why not? Decomposition of POC in the water column can also produce carbon dioxide.
Reply 14:
Thank you for the comment. We agree that biodegradation of suspended POC is a real process that contributes to water-column CO_2 production and that we did not directly measure it in this study. To be precise about what the experimental design captures, the ^{13}C-DIC autotrophy incubations measure the rate of DIC fixation into newly produced suspended POC, observed as ^{13}C incorporation into the filter-retained particulate fraction. The ^{13}C-amino-acid heterotrophy incubations measure two complementary processes within the same incubation: assimilation of labeled DOC into bacterial biomass, captured as ^{13}C-POC on the filter, and respiration of labeled DOC to CO_2, captured as ^{13}C-DIC in the filtrate. What the design does not directly probe is decomposition of pre-existing (^{12}C-dominated) suspended POC to CO_2, which would require either a longer-term incubation tracking $^{12}CO_2$ accumulation or a separate ^{13}C-labeled POC tracer experiment. The measured planktonic heterotrophic rates therefore reflect uptake of labile dissolved organic substrates rather than the full suite of water-column heterotrophic processes.
We have extended the scope-limitation discussion in Section 4.5 to explicitly include suspended-POC decomposition as a parallel pathway not captured by our design. This reinforces the conservative framing already adopted there: our reported planktonic rates likely underestimate total water-column CO_2 production, and the actual contribution of planktonic metabolism to whole-catchment CO_2 evasion is therefore likely greater than the <5% currently reported. As the degradation rates for POC are likely less than for labile DOC, this further supports the conclusion that planktonic metabolism plays a minor role in catchment CO_2 evasion, which is dominated by chemical weathering and petrogenic carbon fixation.

Comment 15:
Line 178: Equation 3 is better split into two equations. Since 'RAA-uptake' and 'RAA-catabolic' are going to be the major results presented, they should be the subjects of the two equations respectively.
Reply 15:
Thank you for the comment. The equation has been split into two separate equations to make the methods clearer. The explanation of the equation has been revised accordingly.

Comment 16:
Line 179: The letter 'R' was already used in Line 174. Please use another symbol.
Reply 16:
Thank you for the comment. We have kept R for the isotope ratio and replaced the metabolic rate R with ρ (rho), which is the standard notation for substrate uptake and production rates in aquatic microbiology. The change has been applied consistently across the three relevant equations including ρ_{DIC_uptake} (Eq. 2), ρ_{AA_uptake} (Eq. 3), $\rho_{AA\_catabolic}$ (Eq. 4).

Comment 17:

Line 211: Specify which statistical tests were conducted. What was the purpose of each test? And what were the variables being used in each test?

Reply 17:

Thank you for the comment. We have revised the Methods paragraph to specify each test, its purpose, and the variables involved.

Briefly, alpha diversity indices (observed ASVs, Chao1, Shannon index) were compared among sites and between seasons using Kruskal-Wallis tests. Beta diversity, based on Bray-Curtis dissimilarity of the normalized ASV table, was tested using permutational multivariate analysis of variance (PERMANOVA, vegan package, 999 permutations) with site and season as predictors. Relationships between community composition and environmental variables were examined by canonical correspondence analysis (CCA), with the significance of each geochemical vector assessed by envfit (999 permutations). The revised statistics paragraph appears in Section 2.4, and the new test results are reported in the Results.

Comment 18:

Line 222: How was each ‘sub-catchment’ defined? How many ‘sub-catchments’ are there? Do they correspond to the five study sites? If so, each study site would represent what area? Please justify why data from one single sampling site could be extrapolated to the entire (sub-)catchment.

Reply 18:

Thank you for these questions. We have revised the content to define “sub-catchment” explicitly and to clarify the basis for catchment-scale extrapolation. Each of the five sampling sites was treated as the outlet of a sub-catchment. Among these five sites or sub-catchments, MLL01 is encompassed within CWL01, while BNE represents the most downstream site of the whole Beinan catchment. Therefore, the area for CWL01 was specified as the whole sub-catchment area minus the area for MLL01. The area for BNE was computed as the whole Beinan catchment area minus the areas of LY04 and mountainous sub-catchment for the main stem. Therefore, the five sub-catchments are: MLL01 (~26 km^2), CWL01 (~600 km^2), DL on Dalun Creek (~300 km^2), LY04 on Luye Creek (~502 km^2), and BNE on the lower main stem (~11 km^2). Together these account for ~1,439 km^2, or 91%, of the 1,584 km^2 Beinan basin; the remaining ~9% corresponds to minor tributaries that were not directly sampled. River surface area for each sub-catchment was assumed to be 0.47% of its catchment area following Raymond et al. (2013). The measured per-site areal flux was then multiplied by the corresponding sub-catchment river surface area to obtain the annual sub-catchment yield. Finally, the total catchment yield was calculated as the sum of the five sub-catchment fields (Table S5).

Regarding extrapolation from a single sampling point, we acknowledge that one site per sub-catchment cannot resolve within-reach heterogeneity. As the flow along the mountainous region is rapid, it is 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. We note this limitation in the revised Methods and discuss its implications in Section 4.5.

Comment 19:

Line 225: Use the singular form after ‘each’.

Reply 19:

Thank you for the comment. The sentence has been corrected to use the singular form after “each” (line 280).

Comment 20:
Line 251: This is the first time that the Wilcoxon test is mentioned. It should have been described earlier in the methods section.

Line 261: Why was the t-test used here? Moreover, what statistical test was used to compare the sites? There were five sites, so neither the Wilcoxon test nor the t-test should be used. In any case, information should have been given in the methods section.

Reply 20:
Thank you for these comments. Indeed, the Wilcoxon test was not described in the original Methods, and a 2-group test on samples pooled across five distinct sites does not appropriately handle the repeated-measures structure of our sampling design.
We have re-analyzed the geochemical data using linear mixed-effects models (R packages lme4 and lmerTest), with season as a fixed effect and site as a random intercept. Strongly skewed variables (TSM, POC, Chl *a*, NH_4^+) were $\log_{10}$-transformed prior to analysis. The corresponding method description has been added to Section 2.3. The revised analysis confirms significant wet-vs-dry differences for water temperature, pH, TSM, POC, δ^{13}C-POC, DOC, Chl *a*, NH_4^+, sulphate, sodium, and calcium (all $p < 0.05$). The original text, Tables 2 and S1 have been updated accordingly.

Comment 21:
Lines 272, 273, 282, 283, 285 to 287, 298 to 300, 306 to 308, 316 to 321: Were statistical tests conducted to evaluate quantitatively where (or when) the measured variables (e.g. rates, biotic indices, relative abundances of the microbial taxa) were higher?
Reply 21:
Thank you for these comments. We agree that several comparative claims in the original Results were not supported by formal statistical tests. We note that Reviewer 2 raised a related concern about the presentation of the metabolic rates in Figure 3 (Comment 15), and the figure has been substantially revised in response to that comment. We have addressed the editor's comments systematically as follows.
For DIC uptake (original lines 272-273, Fig. 3a), among-site differences in August were tested by Kruskal-Wallis (H=18.15, p = 0.001) followed by Dunn's pairwise post-hoc tests, and within-site temporal variation was tested by Kruskal-Wallis at each site. Both sets of test results are now indicated in Fig. 3a (lowercase letters and asterisks, respectively) and referenced in the revised text (lines 358-360). For amino acid uptake and catabolic rates (original lines 282-283 and 285-287; Fig. 3c, d), among-site differences were also tested by Kruskal-Wallis. Glycine uptake did not reach overall significance, but pairwise tests identified BNE as distinct from the other sites, and catabolic rates showed no significance among-site differences (H=5.70, p = 0.23). These results are now stated in Fig. 3 caption and the Results. Pairwise comparisons across time points and substrate (August v.s. January, glycine v.s. leucine) were not tested, because a two-sample rank test with three replicates per group cannot reach statistical significance. The corresponding text has been revised accordingly.
For gene abundance (original lines 298-300; Fig. S2), only a single biological sample was obtained from each site at a specific time. So formal statistical tests on seasonal effect were not feasible. The text has been revised to be descriptive and the limitation is now stated at lines 391-392. For alpha diversity (original lines 306-308; Fig. 4a), Kruskal-Wallis tests on observed ASVs, Chao1, and Shannon indices did not reveal significant differences among sites or between seasons (all $p > 0.08$). The Results text has been revised to state these statistics and to acknowledge that the observed site- and season-level patterns are descriptive trends rather than statistically significant

differences. For phylum-level abundances (original lines 316-321; Fig. 4b), statements comparing phylum abundances between seasons have been revised accordingly.

Although the alpha diversity and phylum-level patterns are now reported as descriptive trends, the community data remain statistically sound. Community composition is significantly structured by site (PERMANOVA, see reply 22) and shaped by significant environmental drivers (CCA with envfit, $p < 0.01$; Fig. 5). The community data therefore can be used to link environmental conditions with metabolic function in the catchment (see also Reply to Reviewer 1, Comment 1).

Comment 22:

Line 323: Can the authors use statistical tests (e.g. PERMANOVA or ANOSIM) to quantitatively compare the microbial communities?

Reply 22:

Thank you for the suggestion. We have added PERMANOVA on the Bray-Curtis dissimilarity matrix to quantitatively compare microbial communities. Site explained a substantial portion of community variation ($F_{4,19} = 2.01$, $R^2= 0.30$, $p = 0.001$), while the seasonal effect was marginal ($F_{1,22} = 1.29$, $R^2 = 0.06$, $p = 0.087$). The joint model (site + season) explained 35% of total variance ($p = 0.001$). These results indicate that microbial community composition along Beinan catchment is structured primarily by site rather than by season.

Comment 23:

Figure 5: Please present statistical results for the significant correlations.

Reply 23:

Thank you for the suggestion. We have updated the Figure 5 caption to report the statistical results for each significant environmental vector. Using envfit on the CCA ordination (9999 permutations, set.seed [42] for reproducibility), four geochemical parameters were significantly correlated with community composition at $p < 0.05$: TSM ($r^2 = 0.94$, $p = 0.006$), POC ($r^2 = 0.96$, $p = 0.007$), DOC ($r^2 = 0.62$, $p = 0.011$), and δ^{13}C-POC ($r^2 = 0.65$, $p = 0.035$).

Comment 24:

Lines 359 to 362: I do not find this discussion necessary. Clearly, organic matter degradation within the water column would not be the only process leading to ammonium input. The discussion should focus on the mechanisms causing ammonium concentrations to be higher during the wet season. Other than high erosion rates, could higher temperatures also lead to faster organic matter degradation? Moreover, did ammonium concentrations increase in the downstream direction? The (lack of) spatial trends should also be discussed.

Reply 24:

Thank you for these comments. We note that Reviewer 2 also commented on this discussion (Comment 17). To reconcile both sets of feedback, the residence-time arithmetic has been removed. We have added a sentence on the temperature mechanism (~6 °C warmer wet-season water) and a sentence describing the downstream gradient in mean ammonium concentration, which rises from 2.6 μM at the headwater site MLL01 to 4.9 μM at the river mouth BNE.

Comment 25:

Lines 385 to 386: New data should not be presented in the discussion. These correlation analyses are important and should be included in the main text earlier.

Reply 25:

Thank you for the comment. The corresponding methods are now stated in Section 2.3 and a summary paragraph reporting the significant correlations ($p < 0.01$) has been added to the Results

The original lines 385-386 in the Discussion have been condensed to a single sentence cross-referencing the Results, so that the subsequent interpretation was based on previously established findings rather than newly presented data.

Comment 26:
Lines 388 to 389: Did any statistical tests help classify DIC rates into those categories? Good that there is an attempt to compare the sites, but the discussion that follows does not completely explain why there was such a difference between the two groups of sites. For example, why did phototrophy contribute to a higher proportion of DIC uptake at CWL01, DL, and BNE? Moreover, the authors should try to compare planktonic and benthic rates here. I suspect that biofilms growing on coarse stream bed substrates at MLL01 and LY04 (upland sites) would exhibit significant rates of photosynthesis.
Reply 26:
Thank you for these comments. We acknowledge that the two-pattern classification of DIC rates was based on visual inspection of Fig. 3a, b rather than a formal statistical test. Formal pairwise tests of light versus dark DIC uptake within each site were not feasible because the dark incubations were not replicated (n=1 per site/season), in contrast to the light incubation (n=3 per site/season). Section 4.2 has accordingly been revised to describe the two patterns as a qualitative grouping. Regarding why phototrophy contributed a larger proportion of DIC uptake at CWL01, DL, and BNE, the contrast is driven by differences in light availability, and we have added a sentence to Section 4.2 stating this. At MLL01 and LY04, the wet-season TSM reached ~157 and ~499 mg L^{-1}, respectively, a level sufficient to substantially attenuate light penetration through the water column (Stanley et al., 2010). At CWL01, DL, and BNE, wet-season TSM was much lower (~4-106 mg L^{-1}), allowing deeper light penetration. Nutrient supply reinforces the same contrast. As reported in Section 4.2 and shown in Fig. S3, the phototrophic component of DIC uptake (light-minus-dark) correlates positively with NO_3^- + NO_2^-, PO_4^{2-}, and DOC (Fig. S3), and these nutrients are highest at the downstream sites. Phototrophy at CWL01, DL, and BNE is therefore favored by both better light availability and higher nutrient supply.
The concern on benthic comparison overlaps with the earlier Comment 4. As detailed in our Reply 4, we have added a literature-based comparison of our planktonic rates with published rates from morphologically comparable upland streams in Section 4.5. The comparison reveals contrasting patterns for autotrophy and heterotrophy. Our planktonic autotrophic DIC uptake is one to two orders of magnitude lower than typical benthic gross primary production for clear upland streams (Hall et al., 2015; Bernhardt et al., 2022), consistent with your expectation that significant photosynthesis at MLL01 and LY04 may occur in the benthic compartment that is not captured by our planktonic incubations. By contrast, our planktonic heterotrophic catabolic rates (0.055-1.85 g-C m^{-2} d^{-1}) fall within the typical range of total ecosystem respiration reported for upland streams. Because ecosystem respiration in those studies integrates planktonic, benthic, and hyporheic contributions, our planktonic-only rates suggest that the planktonic compartment can be a substantial fraction of total stream respiration. Because net catchment CO_2 emission is driven primarily by respiration rather than by photosynthesis, the planktonic compartment may therefore play a more important role in CO_2 evasion than the autotrophy comparison alone would suggest.

Comment 27:
Line 409: Please use 'did not' instead of the abbreviated form in scientific writing.
Reply 27:
Thank you for the comment. The sentence has been modified accordingly (line 505).

Comment 28:

Sections 4.3 and 4.4: Please discuss spatial trends in lithoautotrophy and heterotrophy as well.

Reply 28:

Thank you for the comment. The content in Section 4.3 and 4.4 has been revised, and discuss the spatial trends in lithoautotrophy and heterotrophy. Briefly, Dark DIC uptake was elevated only at the high-sediment-load sites LY04 and MLL01. This pattern is consistent with the control of catchment geology, namely the high erosion of slate formations, rather than with the upstream-downstream position along the river. The spatial patterns of heterotrophy are described as substrate-specific. Glycine uptake is linked to nutrients released by particulate degradation, which accumulate along the flow and peak at the estuary site BNE. Leucine uptake is instead more favorable at the upstream sites, where TSM concentrations are higher and particle-attached populations are larger.

Comment 29:

Line 455: The word ‘expanding’ is incorrectly spelt as ‘expending’.

Reply 29:

Thank you for the comment. The word has been corrected (line 554).

Comment 30:

Line 479: Do the authors mean that a relatively higher proportion was diverted to assimilation during the wet season?

Reply 30:

Thank you for the question. The interpretation is correct. As shown in Table S2 and Fig. S5, the catabolic-to-assimilation ratios were lower in August than in January at most sites, indicating that a relatively higher proportion of the fed amino acids was diverted to assimilation during the wet season compared to the dry season.

Comment 31:

Lines 483 to 484: This is an overstatement because benthic primary production was not considered.

Reply 31:

Thank you for the comment. The remainder of the paragraph already restricts the conclusions to the planktonic compartment, acknowledges that benthic and hyporheic processes were not measured, and includes the comparison with published rates described in Reply 4 (Comment 4). As elaborated in that comparison, planktonic autotrophy is one to two orders of magnitude lower than typical benthic gross primary production in clear upland streams (Hall et al., 2015; Bernhardt et al., 2022), while planktonic heterotrophic respiration falls within the range of total ecosystem respiration reported for comparable upland systems (which integrates planktonic, benthic, and hyporheic contributions). The planktonic compartment may therefore contribute meaningfully to total stream CO_2 production.

Comment 32:

Line 489: Clearly, this study focuses solely on planktonic processes and should not even be taken as an estimate of river metabolism (i.e. not even a conservative one).

Reply 32:

Thank you for the comment. We note that the original sentence at line 489 implied the planktonic measurements were an (conservative) estimate of total river metabolism. We have revised the sentence to make explicit that the current results constrain only the planktonic component and would not be interpreted as an estimate of total river metabolism. The sentence has been revised in Section 4.5. The adjustment is consistent with the scope clarifications in Reply 4 (Comment 4) and Reply 31 (Comment 31).

Comment 33:
Line 490: Planktonic production of carbon dioxide would be what percentage of benthic production? Please provide a quantitative comparison.
Reply 33:
Thank you for the question. A quantitative comparison was added in Section 4.5 in response to the earlier Comment 4. Briefly, our planktonic heterotrophic catabolic rates correspond to 0.055-1.85 g-C m^{-2} d^{-1}, which falls within the typical range of total ecosystem respiration reported for morphologically comparable upland streams (~0.5 to several g-C m^{-2} d^{-1}; Battin et al., 2008; Hall et al., 2015; Bernhardt et al., 2022). Because ecosystem respiration in those studies integrates planktonic, benthic, and hyporheic contributions rather than separating them, a direct planktonic-to-benthic percentage cannot be derived from the literature. However, the fact that our planktonic-only rates fall within the typical total-ER range suggests that the planktonic compartment can constitute a substantial fraction of total stream respiration in such systems. Because net catchment CO_2 emission is driven primarily by respiration rather than by photosynthesis, the planktonic compartment may play a more important role in CO_2 evasion than the autotrophy along would suggest. We note that direct quantitative partitioning at our sites was not possible because benthic rates were not measured in this study.

Comment 34:
Lines 495 to 496: True, but how often was this river system dominated by high flows?’
Reply 34:
Thank you for this question. In typical years, high-flow events driven by typhoons and sustained monsoon precipitation occur frequently throughout the wet season (June to November) in the Beinan catchment, with multiple events per year that can elevate discharge by 5-10× above baseline (Wang et al., 2024). We also note that the 2020-2021 sampling period coincided with an unusually dry year (described in Section 2.1) and therefore captured relatively few high-flow events.

Comment 35:
Lines 496 to 498: This is a speculation not supported by primary data or previous studies.
Reply 35:
Thank you for the comment. The sentence has been revised to remove the speculation and provide quantitative support in Section 4.5. The argument is placed on the order-of-magnitude difference between our measured planktonic CO_2 production and the combined weathering plus petrogenic CO_2 fluxes. This statement is supported by our measurements, weathering and petrogenic fluxes from the cited studies, and by the literature-based benthic comparison in Section 4.5.

Comment 36:
Lines 499 to 514: Comparison with other NEP values does not seem very meaningful as this study only focused on the water column.
Reply 36:
Thank you for the comment. We agree that our value and the published NEP fractions are not strictly comparable. Section 4.5 has been revised to state this limitation explicitly. It notes that the current estimates constrain only the planktonic component of river metabolism, that the benthic and hyporheic zones were not measured, and that the results should not be interpreted as an

estimate of total river metabolism. The value is referred to throughout as a net planktonic CO_2 exchange fraction rather than a whole-stream NEP.

Comment 37:
Line 513: Either 'limit' or 'limited' but not 'limite'.
Reply 37:
Thank you for your comment. The word has been corrected to "limit" in line 648.

Comment 38:
Section 4.6: I recommend integrating this section into Sections 4.1 to 4.4, so that readers can better understand which microbes were responsible for which biogeochemical transformations. Currently, this section reads more like a descriptive results section, and there is insufficient interpretation of why spatial and temporal variations in microbial communities were observed.
Reply 38:
Thank you for this recommendation. We considered carefully whether to integrate Section 4.6 into Section 4.1 to 4.4 and chose to retain Section 4.6 as a unified section. The description on community composition and structure has its own structural logic, organized by site-level patterns and seasonal turnover that are conveyed more coherently as a unified section than when distributed across the process-level sections. Splitting the community discussion into four sections would also substantially lengthen each of those sections and fragment the site-by-site community interpretation. Section 4.6 has also been revised to strengthen the interpretive content. Each site's community pattern is explicitly tied to the corresponding biogeochemical activity. The section also discusses the abundance-activity decoupling observed at MLL01 and LY04 (where lithoautotrophs were numerically abundant during the dry season but dark DIC uptake rates were low). Together, these changes preserve the structural coherence of the discussion on community composition and structure while making the taxa-function links more explicit.

Comment 39:
Conclusions: What were the limitations of this study? Any suggestions for future research? Implications for environmental management? After all, why is it important to understand microbial processes within the water column?
Reply 39:
Thank you for these comments. The Conclusions section has been expanded to address the raised points. Methodological limitations, including the maximum-potential nature of the amino acid-based heterotrophic rates and the single-site representation per sub-catchment, are discussed in the relevant sections of the Discussion. The Conclusions briefly acknowledge them with a cross-reference. Future research directions include high-frequency event-driven monitoring, kinetic experiments to constrain *in situ* substrate uptake, RNA-based approaches to identify actively metabolizing community members, and parallel benthic and hyporheic measurements. From a management perspective, catchment-scale CO_2 budgets in steep mountainous systems are dominated by weathering reactions and petrogenic carbon oxidation rather than by biological metabolism, suggesting that measurement and modeling efforts in such systems should prioritize the geological and chemical pathways. Characterization of the water-column compartment nonetheless remains essential for tracking upstream microbial and geochemical signals through the river system and for predicting downstream fluxes to estuarine environments.

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