Reply on Reviewer Comment 2

Major comment

Comment 1:
The manuscript lacks hypotheses and the introduction and later discussion would benefit from, especially to interlink the different datasets.
Reply 1:
Thank you for the comment. We have restructured the final paragraph of the Introduction to integrate testable hypotheses with the more explicit objectives. Specifically, we hypothesized that i) the seasonal transition to the wet season (characterized by higher temperatures, elevated nutrient availability, and increased particulate organic matter loading) would broadly shift planktonic metabolic activity, particularly phototrophic/autotrophic and heterotrophic production and respiration; and ii) these seasonal hydrological changes would be also reflected in shifts in microbial community composition; iii) in this physically unstable, chronically turbid catchment, frequent sediment mobility would drive temporal variability in planktonic metabolic rates and their relative contribution to the riverine CO_2 evasion.

Comment 2:
The labelling design for the DIC aims to enrich to ~5% of the DIC pool, which is consistent with wider stable isotope probing approaches to provide sufficient enrichment while minimising perturbation of the system by excess increase of the pool. However, the extent of addition for the amino acids does not follow this. A final concentration of 50 µM when the DOC concentration (which is made up of much more than just free amino acids) is between 20-80 µM represents a significant increase in the pool size. While it is acknowledged that rates determined may be stimulated as a result (line 139-140) and I appreciate that rates were still often below detection limits, this very important caveat is lost later in the manuscript. This is especially important to consider when these values are then scaled up to the catchment level but could hugely overestimate actual metabolic fluxes from organic pools. These limitations need to be made much clearer both in the methodology and when you upscale these values to a catchment level.
Reply 2:
Thank you for the comment. The relationship between substrate concentration and microbial activity has been formulated by Michaelis-Menten kinetics (Wright and Hobbie 1966) and applied to numerous laboratory and field studies. In this framework, the uptake or metabolic rate (V) is a saturating function of substrate concentration [S]: $V = V_{max} \cdot [S]/ (K_m + [S])$. K_m is the [S] at which the reaction rate is half of V_{max}. When $[S] >> K_m$, V approaches V_{max} regardless of further increase in concentration, and the community operates near its maximum metabolic capacity. When $[S] << K_m$, V scales nearly linearly with [S] and better reflects possible rates for the target setting. The critical question, therefore, is whether such a relationship and its embedded parameters can be universally applied or valid for most natural settings.
We set out to examine and compare the previously studied examples with our settings. By searching for the literature, we found out that published K_m values for low-molecular-weight organic substrates in freshwater and marine systems are consistently in the sub-µM to low-µM range. For example, Brailsford et al. (2019) reported K_m values of 1.41 µM for amino acid mixtures with a corresponding V_{max} of 0.0004 µmol ml^{-1} h^{-1} (4.8 mg-C m^{-3} h^{-1}) for oligotrophic river water. Boysen et al. (2022) reported half-saturation constant (Kt+S) of 11-79 nM for glycine betaine in oligotrophic North Pacific communities, with V_{max} of 0.36 - 0.56 nM h^{-1} (0.022 - 0.034 mg-C m^{-3} h^{-1}). By applying the relationships for these two settings, our added substrate concentrations (50 µM) well exceeded the K_m values for both cases. Therefore, the projected rates would all reach respective V_{max} values. Our highest uptake rates spanned from 0.49 mg-C m^{-3} h^{-1} for glycine to 0.12 mg-C m^{-3} h^{-1} for leucine, a range falling between these two reported V_{max} values by a great margin. The results suggest that the reported kinetics cannot accurately project our field conditions. Alternatively, our rates were input into the kinetic relationships to derive the possible effective substrate concentrations for the target metabolisms. Since our rates were much greater and less than the V_{max} values for marine and freshwater settings, respectively, such computation practice can only be applied to the freshwater kinetics. Our calculation revealed that the potential effective substrate concentrations were 0.16 µM for glycine and 0.036 µM for leucine. Such a range of substrate concentrations was well below our added concentrations, suggesting that our experimental setting may not stimulate the target metabolic activity as generally thought.

Nevertheless, the comparison between our results with previous studies suggests a likelihood that our amendment of amino acids may not necessarily stimulate microbial activity. However, the comparison also highlights that each system or setting may be inherited with its specific kinetic relationships. A dedicated experiment amended with a series of substrate concentrations and rate measurements is required. We therefore cannot confidently assert that our measured rates approximate true *in situ* fluxes, and we agree with the reviewer that the catchment-scale estimates could be reframed since the experimental constraints could be placed as the maximum potential metabolic rates. That is to say, the relative contribution of water column metabolic rate to the total CO_2 evasion would be even less than our original estimates.
We have incorporated these lines of discussion into the revised form in section 4.5 (lines 541-553) to justify the amended concentration and its implications for the contribution of planktonic metabolic rates to the catchment-scale CO_2 evasion.

Comment 3:
Furthermore, there is no consideration of co-added N for the amino acids (C:N 2:1 glycine; 6:1 leucine) which may also influence the diverging processing of the two amino acids (i.e. if being utilised for N and C is largely being respired, glycine is a much more efficient resource to use than leucine). How the underlying biochemistry may control the observed rates is severely lacking, but important rationale given you select leucine to represent a conservative estimate of heterotrophic activity without any rationale other than it is lower (line 221-222). At points (e.g. Line 444-447), it is implied there is direct uptake but there is no evidence to support this. Finally have the authors considered that only one C position was labelled for both amino acid forms?
Reply 3:
Thank you for the comment raising the consideration of difference in C:N ratio between the two amino acids (2:1 for glycine and 6:1 for leucine). While both molecules provide a single nitrogen atom, glycine provides substantially higher nitrogen-to-carbon density. In principle, this stoichiometric advantage makes glycine a more efficient nitrogen source in nitrogen-limited systems, as bacteria can acquire nitrogen while respiring fewer carbon bonds. However, dissolved inorganic nitrogen concentrations in the Beinan River (TIN: 5.7 - 30.6 μM; NH_4^+: 2.6 - 4.9 μM in average, Table 1) suggest that strict nitrogen limitation is unlikely to be the sole driver of glycine preference in this system. Instead, the observed preference likely reflects the greater metabolic efficiency of glycine-centered pathways: the reductive glycine pathway (rGlyP) supports biomass yields up to 17% higher than other carbon assimilation cycles (Dronsella et al., 2025). Furthermore, the glycine cleavage system (GCS) enables rapid, streamlined energy generation from a simple 2-carbon substrate (Kikuchi et al., 2008). Thus, while favorable C:N stoichiometry may contribute under episodically nitrogen-limited conditions, the metabolic efficiency of glycine metabolism provides a more consistent explanation for the observed preference. We have incorporated this discussion into the manuscript (Discussion section 4.4, lines 508-519).
The contrasting metabolic fate of leucine further supports its use as a conservative baseline for heterotrophic activity. In aquatic microbial ecology, leucine is the standard proxy for bacterial protein synthesis (Kirchman et al., 1985; Kirchman, 2001). As a branched-chain amino acid, leucine catabolism requires at least six enzymatic reactions: transamination to α-ketoisocaproate, oxidative decarboxylation to isovaleryl-CoA, followed by sequential dehydrogenation, carboxylation, hydration, and cleavage steps to ultimately yield acetyl-CoA and acetoacetate for entry into the TCA cycle (Massey et al., 1976; Brosnan and Brosnan, 2006) - requiring substantial enzymatic machinery including biotin-dependent carboxylases and multiple CoA-dependent enzymes. Consequently, bacteria typically prioritize leucine incorporation into proteins rather than respiratory oxidation (Kirchman, 2001). This anabolic preference means that leucine-based rate estimates are inherently conservative relative to glycine. By scaling catchment CO_2 evasion estimates using these lower leucine-derived rates, we provided a minimum boundary for the contribution of planktonic metabolism to the total carbon flux, ensuring the implications were not overestimated by transient responses to high substrate availability. The rationale for choosing leucine has been stated in the Discussion (lines 515-519).
Regarding the labeling position, we are well aware that only the C-1 (carboxyl) position was ^{13}C-labeled for both amino acids (indicated in line 202). The C-1 carboxyl group is typically the first carbon released as CO_2 during initial catabolic steps, including oxidative deamination and decarboxylation reactions (Massey et al., 1976; Kikuchi et al., 2008). Therefore, our ^{13}C-DIC measurements specifically track the initial oxidative decarboxylation potential of these substrates, representing the immediate respiratory capacity of the microbial community. Our ^{13}C-biomass measurements also track incorporation of the C-1 position into cellular material; however, if cells

preferentially remove the carboxyl group before assimilating the remaining carbon skeleton, assimilation rates may represent minimum estimates. Altogether, as glycine is more readily metabolized and carboxyl positioned carbon is efficiently channeled into the production of CO_2, the incubations amended with these two amino acids offer to bracket the possible range of metabolic activity for natural communities. We note that the selection of substrates at specific concentrations for incubations very likely deviates the detection from true rates. In fact, static incubations like this were conducted under conditions different from the field variation and setting at various degrees (e.g., constant luminance intensity and exemption of water flowing so the nutrient and substrate concentrations were not fixed). We were limited to mimic every parameters that may precisely fit in situ conditions. We have clarified the implications of C-1 labeling in the Materials and Methods section (lines 151-152).

Minor comment

Comment 4:
The abstract needs to expand briefly on the rationale behind this work.
Reply 4:
Thank you for the comment. We have expanded the abstract to provide a clearer rationale. We now explicitly state that in high-energy tectonic catchments, physical instability and turbidity likely constrain planktonic metabolic processes, which makes the quantification of contribution of planktonic metabolisms a critical but missing piece of the regional carbon budget.

Comment 5:
Line 11: “issue” is vague – is problem or unknown more precise?
Reply 5:
Thank you for the comment. We have revised the sentence to remove the vague term “issue”.

Comment 6:
Line 15: higher rates of what? Both hetero and autotrophy? Add some values / relative differences of these rates into the abstract.
Reply 6:
Thank you for the comment. We have revised the abstract to clarify what “higher rates” were referred to and to include representative values: Autotrophic DIC uptake rates ranged from 0.03 - 1.98 mg-C m^{-3} h^{-1}, with higher values observed in the wet season. Heterotrophic amino acid assimilation rates were comparatively lower (0.004-0.49 mg-C m^{-3} h^{-1}), yet heterotrophic catabolic rates were dramatically higher than autotrophic rates by one to two orders of magnitude (4.6 - 154.5 mg-C m^{-3} h^{-1}), indicating that the riverine microbial community is strongly heterotrophic and driven primarily by respiratory carbon oxidation rather than biosynthesis. We have revised the abstract to state that heterotrophic catabolic rates exceeded autotrophic carbon fixation rates by one to two orders of magnitude, with both processes showing higher activity in the wet season than the dry season.

Comment 7:
Line 72: states that the Beinan River has some of the highest sediment exports and weathering rates in Taiwan. It would be beneficial to put this on a larger context beyond country-specific for an international journal.
Reply 7:
Thank you for the comment. We have revised the Introduction (lines 80-83) to place the Beinan River in a global context. We highlighted that the sediment yields in this system exceed the global average by one to two orders of magnitude (Hilton and West 2020), characterizing it as a critical global hotspot for land-to-ocean carbon transport.

Comment 8:
Line 74: why were leucine and glycine selected?
Reply 8:
Thank you for the question. Glycine and leucine were selected because they represent dissolved free amino acids (DFAA), the most readily bioavailable fraction of the dissolved organic matter (DOM) pool, which can be directly transported across cell membranes without requiring

extracellular enzymatic hydrolysis (Kirchman, 2001). Beyond this general rationale, these two amino acids were specifically chosen because they represent contrasting ends of heterotrophic metabolism. Leucine is the established standard proxy for bacterial protein synthesis in aquatic microbial ecology (Kirchman et al., 1985; Kirchman, 2001), and its complex catabolic pathway makes it predominantly anabolic, providing a conservative lower bound for heterotrophic activity. Glycine, as the simplest amino acid with a favorable C:N ratio (2:1 vs. 6:1 for leucine), can be rapidly processed through streamlined pathways including the glycine cleavage system (GCS) and reductive glycine pathway (rGlyP) (Kikuchi et al., 2008; Dronsella et al., 2025), making it representative of the more labile, rapidly cycling fraction of the DFAA pool. Together, the two substrates allow us to bracket the range of heterotrophic strategies - from conservative protein synthesis to rapid catabolic turnover - within the riverine microbial community. We have incorporated part of this argument into the text.

Comment 9:
Line 154: Should be ICP-MS
Reply 9:
Thank you for this correction. We have corrected the text (line 177) to use the standard abbreviation ICP-MS.

Comment 10:
Line 178: Formatting of equation is strange
Reply 10:
Thank you for the comment. We have revised the formatting of the equation (line 201) to ensure it follows standard typesetting conventions. The new layout clearly displays the fraction components and the relationship between isotopic enrichment in the various carbon pools, making the calculation of uptake and catabolic rates more intuitive for the reader.

Comment 11:
Line 183-184: Why was the instream δ13C not measured? This is an important end member.
Reply 11:
Organic degradation imparts limited isotopic fractionation on the products, such as DOC and DIC. Therefore, their isotopic compositions resemble those of parental organic matter. In our system, major sources of organic matter are C3 plants and petrogenic organic carbon. Their isotopic compositions ranged from –29 to –25 permil (Lamb et al., 2006) and from –22 to –20 permil (Lien et al., 2025), respectively. Since petrogenic organic matter is more recalcitrant or polymerized, its degradation and conversion to DOC and DIC would be much slower when compared with soil organic matter. Its export to the river would also take a longer and more strenuous path through subsurface rock fabrics and geological structures in metamorphic terranes like in this study. Therefore, only the DOC pool produced from C3 plants is assumed to be linked to the biologically available pool related to this incubation experiment. Based on this, we consider and assume that the isotopic compositions of the DOC pool in our setting would resemble the typical C3 plant.

Comment 12:
Line 250: TSM should be defined at first time of use in main text (only defined in footnote of subsequent table).
Reply 12:
Thank you very much for the comment. We have revised the manuscript (line 278) to define TSM (total suspended matter) at its first occurrence in the main text. This ensures the abbreviation is clearly established before its subsequent use in the Results and Discussion sections.

Comment 13:
Line 254: “smaller” to indicate more negative delta values should be replaced by terminology like “more depleted” as is used in other areas of the MS.
Reply 13:
“Smaller” is referred to the delta values, while “more depleted” is referred to the relative abundances of ^{13}C and ^{12}C in the designated entity. Either way is accepted and suitable in terminology usage. Nevertheless, we have revised this (line 282) to accommodate the convention commonly used by part of the isotope community.

Comment 14:
Line 255-256: This states that ammonium was higher in the wet season than the dry season however this was not significant based on Table 2 – please clarify. If it is the case it is as some sites, they could be bold in the table rather than just the heading to provide these site-specific differences.
Reply 14:
Thank you very much for identifying this discrepancy. This was an error when formatting Table 2. The ammonium concentrations were indeed significantly different between the wet and dry seasons across the catchment, as confirmed by a Wilcoxon test ($p < 0.05$). We have revised the header in Table 2 to accurately reflect this statistical significance.

Comment 15:
Figure 3: due to some very high values, it is very difficult to see the low values in panel b and c; in caption state replication level and what the error bars represent (standard error of the mean? Standard deviation?). Also need to state limit of detection.
The statistical analyses need to be improved as only applied to river chemistry data.
Reply 15:
Thank you for the comments. We have revised Figure 3 accordingly. To better present the low values in panels (b) and (c), we have added insets showing the same data on a logarithmic scale. This allows both the high and low values to be clearly visualized. We also updated the caption to state the replication level (n=3 for all light and amino acid incubations) and to clarify that error bars represent standard deviation. For panel (b), dark incubations were conducted with a single measurement per site per date (n=1). Values below zero were not shown in the figure. The statistical analyses have been conducted for the data, and annotated specifically in the figure. Relevant methodological details, statistical analyses, and interpretation of these patterns have been incorporated in the revised manuscript.

Comment 16:
Line 356: You call this organic matter degradation – but you have only looked at final mineralisation step of these processes. Depolymerisation is generally considered the rate limiting step, so the fluxes quantified only reflect one, generally very rapid, stage of organic matter degradation.
Reply 16:
Thank you for this clarification. We agree that our incubation-derived rates specifically capture the terminal mineralization of dissolved free amino acids rather than the complete organic matter degradation process including depolymerization. The rates cited at line 356 were used as a proxy to support the broader inference of *in situ* organic degradation, which is primarily evidenced by the geochemical signatures in river water - particularly the elevated ammonium concentrations in the wet season - rather than being claimed as direct measurements of bulk organic matter degradation. We have revised the text to clarify that these rates reflect amino acid mineralization specifically, representing one component of the overall heterotrophic degradation process, and that the rate-limiting depolymerization step is not directly constrained by our measurements (lines 395-396).

Comment 17:
Line 359-370: There are a lot of assumptions or suggestions here just for ammonium concentrations which are point measurements only that is limited to determine in situ production especially when high additions of organic nitrogen may influence ammonification rates. The production rates are more potential rather than true so this caveat must be extended to residence times.
Reply 17:
Thank you for the comment. Ammonium concentrations reported here indeed represent point measurements. It can be produced from the degradation of soils and petrogenic organic matter on hillslopes or within the river water. As the transit of river water from upstream to downstream is rapid, the *in situ* degradation for the production of ammonium within river water would be likely limited. Instead, its production and accumulation in the pores of soil and fractures of rock are volumetrically advantageous over the riverine degradative metabolisms. During high water periods, high hydraulic gradients and runoff enable more efficient transport and export of the ammonium pool into the river, enhancing riverine ammonium concentrations. It is also likely that

higher ammonium abundances may stimulate autotrophic ammonium oxidation. However, we do not have definitive evidence to attribute the observed higher DIC rate to the enhanced nitrification activity. The estimate of residence time required to generate the observed ammonium concentration would be even longer if our utilized degradation rates are lower than our measurements. Nevertheless, our quantitative assessment on the potential residence time of in situ production of ammonium from degradation of suspended particulates was based on parameters we collected in this study. While the likelihood of in situ production of riverine ammonium within the river water is low, we are not able to completely rule out this speculation. We have revised the discussion to acknowledge that the residence time calculations are based on the observed production rates and therefore represent lower-bound estimates rather than definitive constraints on *in situ* nitrogen accumulation (lines 402-406).

Comment 18:
Line 401-410: were there any links with the community observed i.e. riverine vs. soil? More primary producers downstream where input from surrounding catchment is relatively less and more processed/recalcitrant therefore rely more on primary producers? Currently only consider the nutrients as a control here but community may also reflect this and provide additional support to this suggestion.
Reply 18:
Thank you for the comment. We agree that riverine organic matter downstream may have experienced multiple processes of degradation along the transit. Therefore, they would become more recalcitrant, precluding themselves from further utilization or respiration. Under this context, heterotrophy downstream could be better sustained with the proliferation of *in situ* phototrophy in downstream. Examination of the 16S rRNA gene community data (Fig. 4b) reveals that BNE is predominantly characterized by heterotrophic taxa - particularly Bacteroidota, Firmicutes, and Gammaproteobacteria - throughout most sampling periods, rather than showing elevated abundance of primary producers such as Cyanobacteria. Therefore, it would be challenging to directly attribute such a DNA-based community composition to the stimulation of phototrophic activity driven by the limited accessibility of recalcitrant organic matter for heterotrophy. We acknowledge that the potential disconnect between community composition and measured rates at BNE - as noted in Section 4.6 - suggests that numerically abundant community members are not equivalently metabolically expressed, and that RNA-based analyses would be needed to identify the truly active phototrophic populations. Regarding the potential influence of soil-derived vs. riverine communities and upstream inputs from agriculture and human activity, while these are plausible contributors to the nutrient accumulation pattern observed at BNE, our current dataset does not include sufficient spatial resolution of terrestrial inputs to quantitatively constrain these contributions. We noted this as a direction for future investigation.

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