

Roles of planktonic metabolism in CO₂ fluxes along the river-estuary continuum in a rapidly uplifting catchment of eastern Taiwan

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Abstract. The contribution of river metabolism to carbon cycling remains poorly examined in the catchments susceptible to the modulation of active tectonics. In these high-energy mountainous systems, chronically high turbidity and frequent sediment mobility create an unstable fluid regime that may impact planktonic metabolism, shaping the functional expression for aquatic carbon transformation. Using the Beinan River in southeastern Taiwan as a model system, this study aims to quantify the potential rates of planktonic autotrophy and heterotrophy, and to identify the potential community members involved in these processes. To address this, river water samples were collected across seven campaigns spanning wet and dry seasons for incubations with ¹³C-labeled dissolved inorganic carbon and amino acids. Our results revealed that metabolic rates were consistently higher in the wet season than in the dry season, with heterotrophic catabolic rates exceeding autotrophic carbon fixation by one to two orders of magnitude, indicating strong net heterotrophy. The obtained rates were further scaled up, resulting in the catchment-scale CO₂ evasion of 7.89 × 10⁷ mol yr⁻¹, a range constituting about 3.8% of the CO₂ flux derived from pyrite-induced carbonate weathering, oxidation of petrogenic carbon, and the river-air exchange. The microbial community structures varied seasonally at upstream sites, with more abundant chemolithoautotrophic sulfur- and ammonia/nitrite-oxidizing taxa in the wet season, as opposed to more abundant phototrophs or heterotrophs in the dry season. This study highlights that even in oligotrophic, physically dynamic mountainous systems prone to rapid uplift and erosion, planktonic metabolisms constitute a measurable and vital component of the riverine carbon cycle.

1. Introduction

Inland freshwater systems release CO₂ to the atmosphere at a flux (0.65–2.1 Pg C yr⁻¹) comparable with that of ocean CO₂ uptake (2.2 Pg C yr⁻¹) (Horgby et al., 2019; Lauerwald et al., 2015; Takahashi et al., 2009). The magnitude is enormous given their limited area coverage of the global land surface (0.3–0.56%) (Allen and Pavelsky, 2018; Aufdenkampe et al., 2011). Among a variety of inland sub-systems, mountainous catchments, which comprise 27% of the Earth's land surface (Ives et al., 1997) and drain more than 30% of the global runoff, receive far less attention when compared with low-altitude catchments in temperate regions (Bandyopadhyay et al., 1997; Meybeck et al., 2001; Viviroli and Weingartner 2004). While the accessibility to sampling or measuring in such high-relief catchments is often limited, a few previous studies have projected that CO₂ evasion declines substantially by orders of magnitude as a function of distance from the headwater (Raymond et al., 2013; Schelker et al., 2016; Chiriboga and Borges 2023). Such a change of flux is intrinsically tied to the summed effect of P_{CO2} (CO₂ partial pressure) and exchange coefficient. Even fewer studies have further demonstrated that mountainous rivers draining the catchments composed of crystalline rocks are

characterized by P_{CO_2} slightly exceeding those in equilibrium with the atmospheric CO_2 (Horgby et al., 2019). Therefore, the enhanced CO_2 evasion in the upstream region could be largely attributed to the steep topography, where turbulent flow and rough river bed enable the rapid exchange of CO_2 between the air and water. As the river slope decreases toward the downstream region, the degree of topographically controlled CO_2 evasion diminishes gradually. The pattern highlights the role but also points out the deficiency in data coverage for mountainous catchments in configuring the global carbon inventory.

A number of factors have been considered to contribute to the overall pattern of CO_2 evasion along the river corridor. Among these factors, groundwater input and *in situ* microbial processes are often considered the most important source mechanisms (Brunke and Gonser 1997; Cole et al., 2007; Marx et al., 2017). Measurements mostly using dissolved oxygen sensors have shown that aquatic metabolisms contributed to a sum of 28% of the CO_2 evasion for streams and rivers across the US continent (Butman and Raymond 2011; Hotchkiss et al., 2015). A few other studies have further demonstrated that benthic and hyporheic compartments contribute significantly to the whole stream metabolism in many upland systems, particularly when water clarity permits light to reach coarse streambed sediments (Hall et al., 2015; Battin et al., 2023; Bernhardt et al., 2022; Roche et al., 2025). For mountainous catchments, most studies intuitively assume that groundwater input would dominate over *in situ* aquatic microbial processes in a way that the catchments are composed of exposed bedrocks rather than thick soils accumulated over a prolonged period of weathering and that the residence time of substrates is perhaps too short to be made available for microbial degradation (Marx et al., 2017; Duvert et al., 2018). Therefore, the quantities of the products derived from soil degradation (presumably attributed to dissolved organic carbon) would be scarce, thereby rendering the limited availability of substrates for microbial respiration. While the terrestrially derived fluxes (equivalent to lateral transport of groundwater) may dominate the CO_2 evasion in small streams, the percentage of aquatic metabolic flux to the overall evasion is postulated to increase with stream size (Hotchkiss et al., 2015). The exact fraction of metabolic flux to the overall evasion in mountainous rivers remains largely unconstrained.

The observations of diurnal variation in dissolved oxygen represent the net outcome combining primary production and ecosystem respiration. Therefore, the contribution of individual categories of metabolisms and further extrapolation to the CO_2 variation could only be deconvoluted with the computational scheme inherited with assumptions (Appling et al., 2018; Saccardi and Winnick, 2021). Furthermore, whether observations based on the cratonic continent or large river systems could be applicable to other catchments around the world remains less constrained. In particular, small island catchments susceptible to active tectonics are constantly impacted by torrential precipitation associated with monsoons and typhoons, mass wasting processes, and structurally controlled transport of groundwater (McMillan and Srinivasan 2015). The event-based hydrological and geological impacts would have led to a pattern of nutrient and substrate availability (e.g., turbid river water during high water) and transport of microbial populations fundamentally distinct from the major river systems. As frequent sediment mobility, bed scouring, and episodic turbidity pulses may impact planktonic processes across the full range of hydrological conditions, dissecting the relative contributions of individual planktonic metabolic pathways (phototrophy, chemoautotrophy, and heterotrophy), provides a necessary first step toward resolving the role of planktonic compartment within the broader river carbon-cycling network.

In this study, we aimed to quantify planktonic metabolic rates and characterize microbial community dynamics along the Beinan River. As a representative of high-standing island catchments, the Beinan River is characterized by high sediment exports and weathering rates that rank among the greatest in Taiwan (Dadson et al., 2003) and exceed the global average by orders of magnitude (Hilton and West, 2020). To quantify metabolic activity, we performed short-term incubations

supplemented with ^{13}C -labeled dissolved inorganic carbon (DIC) and amino acids (leucine and glycine) to constrain assimilation and respiration rates of different metabolic pathways, complemented by 16S rRNA gene amplicon sequencing to characterize microbial community composition. We tested three interrelated hypotheses: (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 activities; (ii) these seasonal hydrological changes would be reflected in shifts in microbial community composition; and (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. By testing these hypotheses with direct ^{13}C -based rate measurements and community composition data, this study seeks to provide a mechanistic framework for understanding how physical disturbance, seasonal hydrology, and microbial structure interact to shape carbon cycling in tectonically active mountainous rivers.

2. Materials and methods

2.1 Field background

Taiwan was formed through dual subduction and interactions between the Eurasian and Philippine Sea Plates (Teng 1990). The northwestward impingement of the Luzon arc on the Eurasian continent since 5 Ma has created steep topographic changes with the unroofing of metamorphic complexes in the backbone range, from which major rivers originate (Chen et al., 2019). Across the island (particularly central and southern Taiwan), rapid uplift (up to several centimeters per year), river incision, event-based torrential precipitation, and frequent earthquakes have generated numerous landslides with sediments transported along with flow or temporarily deposited in the riverbank/bed and subsequently remobilized during the high-water period (Dadson et al., 2003; Kao and Milliman, 2008). The Beinan River, which is one of the largest rivers in Taiwan in terms of discharge and suspended sediment load (Dadson et al., 2003; Kao and Milliman, 2008; annual report of the Water Resource Agency, MOEA), flows eastward across the central mountain and drains southward along the Longitudinal Valley prior to reaching the ocean. The catchment covers an area of $\sim 1600 \text{ km}^2$ and is primarily composed of schist and slate, with minor amounts of marble and meta-sandstone in the mountain part (Stanley et al., 1981). The soil development is limited and hindered by high erosion rates. Therefore, detritus materials that are susceptible to pulverization and abrasion are primarily composed of rock fragments. Land cover in the catchment is dominated by natural and semi-natural vegetation. Evergreen broadleaf and mixed montane forest cover the majority of the upper and middle catchment, with exposed bedrock, landslide scars, and riverbed gravel deposits accounting for a non-trivial fraction consistent with the high denudation regime of the region. Human activity and land use are limited and concentrated in the lower Longitudinal Valley and the coastal alluvial plain near the estuary. Population density in the catchment is low relative to western Taiwan, and no large impoundment exists on the main stem. A land-use and land-cover overview of the lower catchment is shown in Fig. 1b.

Sampling sites were selected to represent individual sub-basins of the Beinan catchment and to span the elevation gradient and landscape transition from metamorphic headwater tributaries to the estuary (Fig 1a and S1a). Among these sites, MLL01 is located in an upper metamorphic-headwater tributary in the forested headwaters and is characterized by a steep, boulder- and cobble-dominated channel within a valley. CWL01 is on the upper main stem of the Beinan River and DL is on an adjacent mid-catchment tributary that merges with the main stem near CWL01; both sites are characterized by coarse-grain beds and extensive exposure of gravel/sand bars during low flow. LY04 is on a lower tributary, where the channel broadens into a wider, cobble-bed reach. BNE is on the Beinan main stem at the river mouth. Field photographs of all five sites are provided in Fig. S2. *In situ* surface flow velocities measured during seven sampling campaigns ranged

120 from 0.3 to 2.5 m s⁻¹ (Table S1), reflecting the high-energy characteristics of the catchment. At BNE, *in situ* salinity
121 remained below 0.6 ppt in every campaign, and dissolved Cl⁻ and Na⁺ concentration (0.22–0.28 mM and 0.65–0.77 mM,
122 respectively; Table S1) were several orders of magnitude lower than seawater values (~550 mM Cl⁻, ~470 mM Na⁺) and
123 comparable to those at upstream freshwater sites, indicating that seawater intrusion was negligible during the sampled
124 conditions and that BNE should be interpreted as the freshwater end of the river-estuary continuum rather than a fully
125 mixed estuary.

126 Previous studies based on carbon isotopic compositions and radiocarbon contents have inferred variable contributions of
127 soil to particulate organic matter (Hilton et al., 2008; Lin et al., 2020). Monsoon combined with typhoon from summer to
128 autumn contributes the majority of precipitation (generally >3000 mm) and runoff in the upstream region. For this study,
129 the wet season was referred to the period between June and November 2020 during which the precipitation accumulated
130 substantially (Fig. S1). Notably, samples collected in May is referred to as the dry season considering that the field
131 campaign in May of 2020 was conducted prior to the significant increase in precipitation in late May (Fig. S1). It is also
132 worth noting that the sampling period represents the driest year (annual precipitation of 1856 mm) over the last 20 years.

133 2.2 Sampling and field experiments

134 Seven field campaigns were conducted in January, March, May, July, August, and October 2020 and January 2021 (Table
135 S1). This frequency was chosen to provide a systematic baseline that spans the full hydrologic cycle, encompassing the
136 distinct dry (January – May) and wet (August) seasons characteristic of southeastern Taiwan (Fig. S1). The intervals also
137 allow for a consistent comparison of metabolic rates across seasonal transitions, providing a framework to assess the long-
138 term metabolic stability of these dynamic turbid systems. While *in situ* environmental parameters were characterized in
139 all campaigns, metabolic rates were measured in March and May 2020 (for phototrophy and autotrophy only) and in
140 August 2020 and January 2021 (for the full suite of tested metabolisms). At each site, *in situ* physicochemical
141 characteristics (temperature, pH, conductivity, and river flow rate) were measured using the portable probes and meters
142 (Hanna Instruments, HI-98130, RI, USA; Pro II SVR, Stalker, TX, USA). Samples were collected in different forms with
143 different preservatives and preparations (Wang et al., 2024). In brief, river water was filtered using sterilized 0.22 µm
144 pore-size polyethersulfone (PES) membrane (Supor®) and collected for analyses of dissolved inorganic carbon (DIC),
145 major cations and anions, dissolved organic carbon (DOC), and dissolved inorganic nutrients. To minimize carbon and
146 trace element contamination, all syringes and high-density polyethylene (HDPE) bottles for DOC and cation samples
147 were pre-washed through a rigorous cleaning protocol consisting of acid-wash with concentrated nitric acid followed by
148 triple-rinsing with both Milli-Q water and the filtered river water before final collection. Cation samples were acidified
149 with 1/10 volume of 20% nitric acid (Baseline grade, Seastar, BC, Canada). Samples were stored at room temperature
150 (for DIC and inorganic solutes) or –20 °C (for DOC and nutrients). Particulate samples were collected on 0.3 µm pore-
151 size glass fiber membranes pre-combusted at 500 °C for 6 hours to remove any potential organic contaminants. The total
152 volume of filtrate for individual particulate samples was 1 L. The collected membranes were frozen at either –20 °C for
153 analyses of chlorophyll *a* (Chl *a*) and abundances and isotopic compositions of particulate organic carbon (POC), or –80
154 °C for molecular analyses.

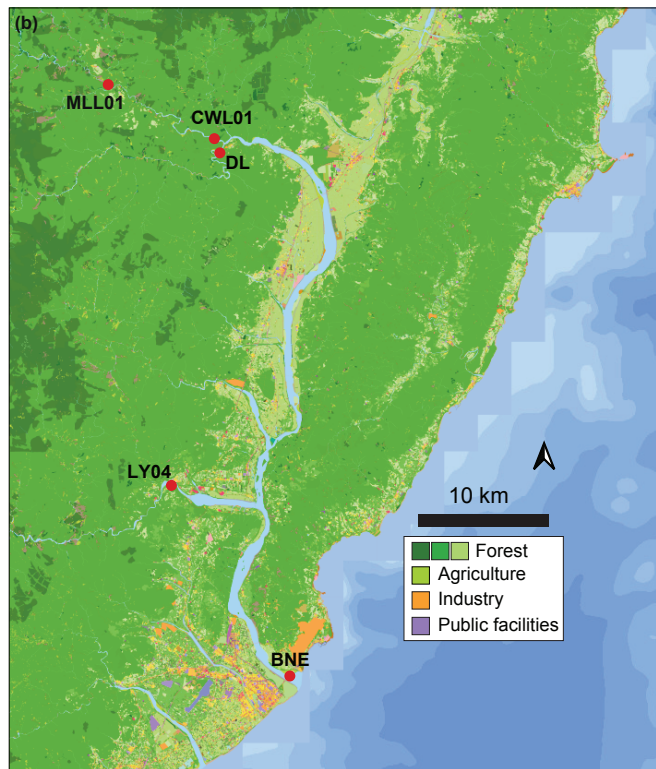
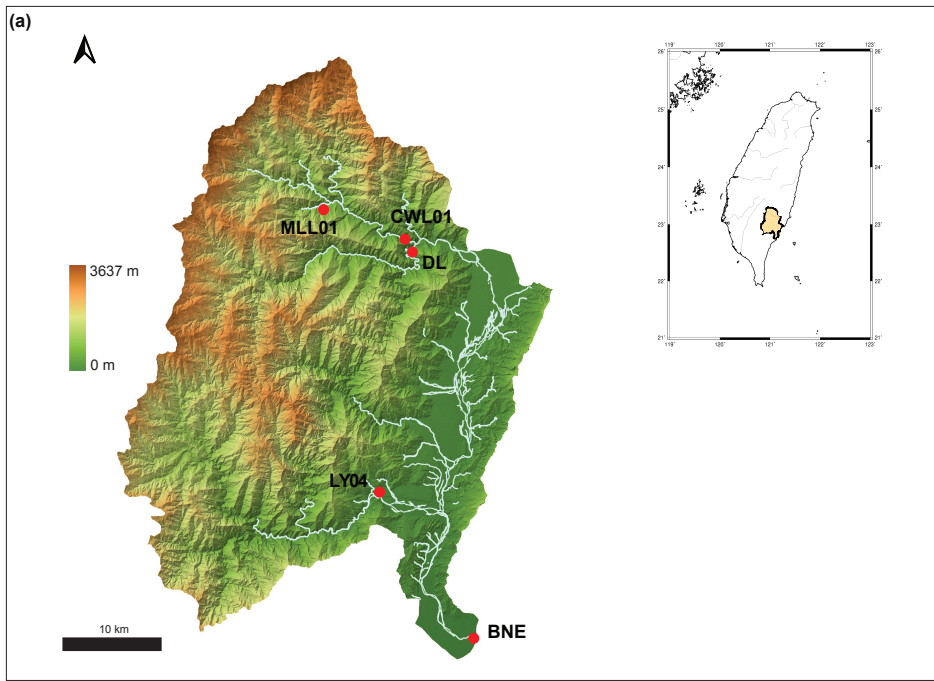


Figure 1: (a) Topographic map of the Beinan River catchment (eastern Taiwan) with sampling sites marked in red. (b) Land use and land cover of the lower Beinan catchment, derived from the National Land Use Investigation dataset (National Land Surveying and Mapping Center, Ministry of the Interior, Taiwan).

For metabolic rate measurements, river water was collected into a water bag pre-washed with river water several times, and supplemented with ^{13}C -labeled bicarbonate and individual amino acids ($[1-^{13}\text{C}]$ glycine or $[1-^{13}\text{C}]$ L-leucine; ^{13}C labeled at the C-1 [carboxyl] position) at a final concentration of $50\ \mu\text{M}$ to constrain the rates of autotrophy/phototrophy (using DIC) and heterotrophy/respiration (using amino acids), respectively. The supplemented substrates with a ^{13}C purity of $>99\%$ were mixed with deionized water to generate a stock at a concentration of $1\ \text{mM}$. The stock for amino acids was acidified by titrating with HCl to $\text{pH} < 4$ so the potential DIC associated with the dissolution of the amino acid powder

could be removed. The stock of either DIC or amino acid was filter-sterilized and stored at 4 °C until further use in the field.

Six liters of river water were collected for each substrate type. All the incubation experiments were conducted in the field accommodation during each campaign. The supplemented river water was dispensed into 1 L HDPE bottles, completely filled to eliminate headspace, and incubated for 4 to 6 hours. For ^{13}C -DIC autotrophy, three transparent (light) bottles and one opaque (dark) bottle were used per site per campaign. The light bottles were incubated under constant illumination ($\sim 18,000$ lux, monitored by HOBO, Pendant MX) and placed in a flowing tap-water bath at the field accommodation, by which heat from the lighting unit was dissipated to keep the bottles at near-ambient temperature. The dark bottle was incubated alongside. ^{13}C -amino-acid heterotrophy incubations (glycine and leucine, three replicate bottles each) were conducted in opaque bottles at ambient temperature. The incubated samples were filtered using $0.3\ \mu\text{m}$ pore-size glass fiber membranes to collect particulates that represent a sum of mineral/rock fragments and microorganisms. The membranes were rinsed with 2 N HCl immediately after the filtration to remove carbonate minerals and then washed with sterilized saline solution. The collected membrane was stored at $-80\ ^\circ\text{C}$ for further isotopic and molecular analyses. Part of the filtrates for the incubations with amino acids were preserved and stored at room temperature for isotopic analyses of DIC. A conceptual diagram describing the experimental setup is shown in Fig. 2.

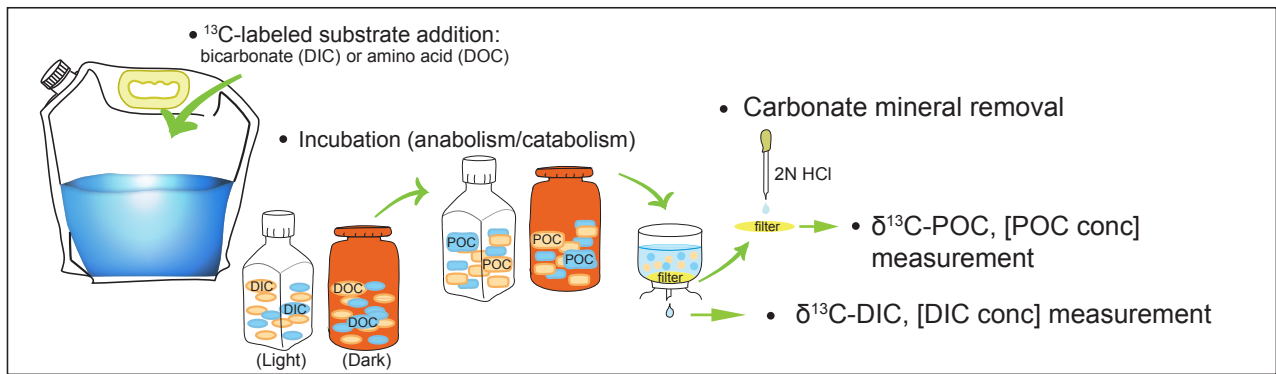


Figure 2: Conceptual diagram for the incubation setup in fields and following laboratory measurements.

2.3 Geochemical analyses

Anions were measured using an ICS-3000 ion chromatography (Thermo Fisher Scientific, USA). Major cations were analyzed using an inductively coupled plasma linked with a quadrupole mass spectrometry (ICP-MS; 7800, Agilent Taiwan, Taiwan). Synthetic and river water standards (SLRS-5 from the National Research Council of Canada) were used to calibrate a range of cation concentrations. Concentrations of orthophosphate and ammonium were measured on molybdenum blue generated from the reductive reaction between phosphate complex and ascorbic acids (APHA Method 4500-P, 2005), and indophenol generated from the reaction between ammonium and thymol (APHA Method 4500-NH₃, 2005), respectively. The intensity of the color complex was quantified using a spectrophotometer (V-500, Jasco, MD, USA). Nitrite/nitrate concentrations were quantified using a commercial fluorometric assay (Cayman, MI, USA), which reacts with nitrite (*in situ* or reduced from nitrate) with 2,3-diaminonaphthalene for the generation of 1(H)-naphthotriazole (Miles et al., 1995). Chlorophyll *a* (Chl *a*) was extracted from membranes with acetone under dark conditions and quantified using a fluorometer (10-AU, Turner Designs, CA, USA). DOC concentration was measured using a Shimadzu TOC-L CPH/CPN system with high-temperature catalytic oxidation pre-calibrated with known concentrations of KHP (potassium hydrogen phthalate). The analytical uncertainty for these methods is less than 2%. Concentrations and carbon isotopic compositions of DIC were analyzed by acidifying samples with concentrated phosphoric acid (85%) and purging

the produced CO₂ into a total carbon analyzer (Aurora 1030, OI Analytical, USA) and an isotope ratio mass spectrometer (IRMS; MAT253, Thermo Fisher Scientific, USA) connected with a GC-IsoLink (Thermo Fisher Scientific, USA), respectively. Concentrations and carbon isotopic compositions of particulate organic carbon (POC) were analyzed on freeze-dried, acid-rinsed membranes using an elemental analyzer (EA; MICRO cube, Elementar, Germany) and an IRMS (Delta V) coupled to a Flash EA (Thermo Fisher Scientific, USA), respectively. Carbon isotopic compositions were expressed as the delta notation:

$$\delta^{13}\text{C} = \left[\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right] \times 1000 \text{ ‰} \quad (1)$$

where R is the ratio of ¹³C to ¹²C, and the standard is Vienna Pee Dee Belemnite (VPDB). The uncertainties for DIC and POC concentrations and $\delta^{13}\text{C}$ values were less than 2% and 0.2‰, respectively.

Metabolic rates were calculated using the following equations:

$$\rho_{\text{DIC_uptake}} = \frac{\Delta F^{13}\text{C}_{\text{POC}}}{F^{13}\text{C}_{\text{DIC}}} \times [\text{POC}] \times \frac{1}{t} \quad (2)$$

$$\rho_{\text{AA_uptake}} = \frac{\Delta F^{13}\text{C}_{\text{POC}}}{F^{13}\text{C}_{\text{DOC}}} \times [\text{POC}] \times \frac{1}{t} \quad (3)$$

$$\rho_{\text{AA_catabolic}} = \frac{\Delta F^{13}\text{C}_{\text{DIC}}}{F^{13}\text{C}_{\text{DOC}}} \times [\text{DIC}] \times \frac{1}{t} \quad (4)$$

where $\rho_{\text{DIC_uptake}}$ is the rate of DIC assimilated into biomass (Eq.2), $\rho_{\text{AA_uptake}}$ is the rate of amino acid assimilated into biomass (Eq. 3), and $\rho_{\text{AA_catabolic}}$ is the rate of amino acid respired to DIC (Eq.4). $F^{13}\text{C}$ represents the fraction of ¹³C in POC, DIC, or DOC; $\Delta F^{13}\text{C}$ is the change in $F^{13}\text{C}$ of POC or DIC during the incubation (post-incubation value minus the T0 reference value); [POC] and [DIC] are the measured POC and DIC concentrations, respectively; and t is the duration of incubation. The isotopic fractionation associated with the target metabolic reaction is assumed to be negligible. In this regard, the $F^{13}\text{C}_{\text{DIC}}$ in eq. 2 and $F^{13}\text{C}_{\text{DOC}}$ in eqs. 3 and 4 represent the sum of the ¹³C fractions of added substrate and original forms (DIC or DOC). The $\delta^{13}\text{C}$ values of *in situ* DOC were not available and, therefore, assumed to be -27‰ considering the isotopic compositions of soil organic matter are in a similar range (Wang et al., 2024; Lien et al., 2025). The rate for the incubation with DIC under light is often considered to represent the summed rate of phototrophy and lithoautotrophy, and could be greater than the rate for the dark incubation. The difference is generally attributed to the phototrophic rate, assuming that the true lithoautotrophy is not affected by light illumination. For incubations with labeled DIC, the chosen concentration constituted a minor fraction of the environmental DIC pool (<5% of the environmental DIC; Wang et al., 2024). For incubations with labeled amino acid, the 50 μM addition represents a potential enrichment relative to ambient dissolved free amino acid concentration in oligotrophic systems (typically < 0.1 μM; Jørgensen and Søndergaard 1984; Raymond and Spencer 2015) and is comparable to the total ambient DOC concentration (20–80 μM). In this context, the measured autotrophic and phototrophic rates based on DIC labeling are considered representative of *in situ* rates, whereas the measured amino acid assimilation and respiration rates represent potential heterotrophic activity or upper bounds rather than *in situ* rates.

Seasonal differences in environmental parameters were assessed using linear mixed-effects models (R packages lme4 and lmerTest), with season as a fixed effect and site as a random intercept to account for repeated sampling. Strongly skewed variables (TSM, POC, Chl *a*, NH₄⁺) were log₁₀-transformed prior to analysis to satisfy the normality assumption on residuals. Among-site differences in metabolic rates were assessed using Kruskal-Wallis tests followed by Dunn's post-hoc tests. Within-site seasonal variations were evaluated using Kruskal-Wallis tests (multiple time points) or Mann-

Whitney U tests (pairwise comparisons). Relationships between rates and environmental variables were examined using Pearson correlations. All the statistical analyses were performed using R version 4.4.1.

2.4 Molecular analyses of community compositions

Crude DNA was extracted and purified using the DNeasy PowerSoil kit (Qiagen, USA) following the manufacturer's protocol, and stored at -80°C for further analyses. Polymerase chain reaction (PCR) amplification was conducted on all extracts using the primers 515F (GTG CCA GCM GCC GCG GTA A) and 806R (CCC GTC AAT TCM TTT RAG T), which target the V4 region of the 16S rRNA gene (Kozich et al., 2013). The amplicons were purified, pooled, barcoded, and sequenced using the Illumina MiSeq platform (Illumina, USA). The detailed methodology and reagents employed for PCR have been described in Tu et al. (2017, 2022). Due to the insufficient quantity of DNA (<50 ng in total), extracts for incubated samples were not further processed for stable isotope probing analysis, which targets the population members actively assimilating the fed ^{13}C -labeled substrate.

Quantitative PCR (qPCR) was performed to determine the copy numbers of bacterial and archaeal 16S rRNA genes and genes encoding ribulose-1,5-bisphosphate carboxylase/oxygenase forms I (*cbbL*) (Alfreider et al., 2003) using the QuantStudio TM 3 Real-Time PCR System (Applied Biosystems, ABI, USA). The standard preparations and PCR conditions were as described in Tu et al., (2017) and Wang et al., (2014). The primers and annealing temperatures for the different genes are summarized in Table 1. The copy number was calculated using the threshold cycles calibrated against standards with known concentrations of gene amplicons, assuming 650 g mol^{-1} for DNA molecules.

Raw sequences of 16S rRNA gene amplicons were processed using Mothur ver. 1.48.0 (Schloss et al., 2009) and QIIME2 (Bolyen et al., 2019). The individual reads were quality-filtered, denoised, merged, and dechimerized to generate Amplicon Sequence Variants (ASVs). Taxonomic classification was performed using the SILVA database (version 138) (Quast et al., 2012). Sequence reads were rarefied through 100 sequence random re-sampling (without replacement) to account for the difference in sequencing depth. Alpha diversity indices were calculated, including observed ASVs and Chao1 for richness (Chao, 1987) and Shannon index for evenness (Shannon and Weaver 1949). Differences in alpha diversity among sites and between seasons (wet, dry) were tested by Kruskal-Wallis tests. For beta diversity, the entire ASV table was normalized and Bray-Curtis dissimilarities were computed. Differences among sites and seasons were tested by permutational multivariate analysis of variance (PERMANOVA; adonis2 in package vegan, 999 permutations). Relationships between microbial community composition and environmental variables were examined by canonical correspondence analysis (CCA) (González et al., 2008), with the significance of each environmental vector assessed by envfit (vegan, 9999 permutations; $p < 0.05$). All statistical analyses were performed using R version 4.4.1 with the vegan, ggplot2, and phyloseq packages. The obtained sequences were deposited in GenBank (BioProject ID: PRJNA1270665).

267

Table 1: Primer information in this study.

Primer name	Sequence (5'-3')	Target group/gene	Annealing temp (°C)	Reference
B27F	AGAGTTTGATCMTGGCTCAG	Bacteria/16S rRNA	55	Lane (1991)
Eub338R	GCTGCCTCCCGTAGGAGT	Bacteria/16S rRNA	55	Amann et al. (1990)
U1492R	GGTTACCTTGTTACGACTT	Bacteria/16S rRNA	55	Lane (1991)
A8F	TCYGGTTGATCCTGCC	Archaea/16S rRNA	55	Huber et al. (2002)
A806F	ATTAGATACCCSBGTAGTCC	Archaea/16S rRNA	55	Takai and Horikoshi (2000)
A958R	YCCGGCGTTGAMTCCAATT	Archaea/16S rRNA	55	Hinrichs et al. (1999)
U1513R	ACGGHTACCTTGTTACGACTT	Archaea/16S rRNA	55	Huber et al. (2002)
cbbLR1F	AAGGAYGCGAGAACATC	autotroph/ <i>cbbL</i>	58	Selesi et al. (2007)
cbbLR1intR	TGCAGSATCATGTCTTT	autotroph/ <i>cbbL</i>	58	Selesi et al. (2007)

268

269 2.5 Computation for catchment scale CO₂ fluxes

270 To scale planktonic metabolic rates to the catchment level, the five sampling sites were treated as the outlets of five sub-
271 catchments, with boundaries and areas taken from the Water Resource Agency (WRA) hydrological atlas of the Beinan
272 basin (Table S5). Measured rates were multiplied by the average water depth of each investigated reach (50 cm for
273 tributaries, 70 cm for main stem) to obtain areal fluxes. These depth estimates reflect typical conditions observed during
274 field campaigns, excluding flash flood periods. DIC uptake rates under light and dark conditions were assumed to
275 represent daytime and nighttime rates, respectively, with total daily DIC uptake calculated as the sum of equal
276 contributions from each. Using leucine incorporation rates as a conservative estimate of heterotrophic activity, net CO₂
277 production for each sub-catchment was calculated by multiplying the per-site area flux with the sub-catchment river
278 surface area, taken as 0.47% of its sub-catchment area (Raymond et al., 2013). The total catchment yield was obtained as
279 the sum of the five sub-catchment yields.

280 To compare the metabolic fluxes with the CO₂ evasion, CO₂ exchange fluxes between river water and the atmosphere
281 were calculated for each sub-catchment using the following equation:

$$282 \text{ Flux} = k_{\text{CO}_2} \times \Delta (C_{\text{measured}} - C_{\text{eq}}) \quad (5)$$

283 where k_{CO_2} is the gas exchange velocity, and C represents the partial pressure of CO₂ either from measurement or in
284 equilibrium with the atmospheric CO₂ concentration (assumed to be 410 ppm). C_{measured} was derived from the DIC
285 concentration, pH, salinity, temperature, and total atmospheric pressure measured in the same field campaign using co2sys
286 (Lewis and Wallace, 1998). k_{CO_2} was computed using the relationships reported by Ulseth et al. (2019), where k_{600} values
287 vary positively with eD (energy dissipation rate and is equal to the product of g [gravitational acceleration], v [flow
288 velocity] and s [slope of the reach]). Depending on whether the gas exchange is controlled by turbulence or bubble, the
289 relationships between k_{600} and eD are different (Ulseth et al., 2019):

$$290 \ln k_{600} = 6.43 + 1.18 * \ln eD \text{ (for } eD > 0.02) \quad (6)$$

$$291 \ln k_{600} = 3.10 + 0.35 * \ln eD \text{ (for } eD < 0.02) \quad (7)$$

292 The flow velocity was adopted from the measured ones obtained from the field, which represented the maximum velocity
293 on the river surface. Because friction would be substantial near the channel bed, the measured surface velocity would be
294 higher than the average channel velocity integrated over the channel cross section. However, considering the water depth
295 is generally small, the measured flow velocity may not substantially deviate from the true one. Nevertheless, the calculated
296 eD values would represent an overestimate. Slope was calculated for the investigated reach (~1000 m in length) using the
297 DEM (digital elevation model) available for the public (data.gov.tw). All the parameters used for calculation were
298 summarized in Table S6. The obtained k_{600} value was further converted to k_{CO_2} using the following equation (Wanninkhof,
299 2014):

$$300 k_{\text{CO}_2} = k_{600} / [(600 / Sc_{\text{CO}_2})]^{(-n)} \quad (8)$$

301 where Sc is Schmidt number of CO₂ at the *in situ* temperature and n is assumed to be 0.5 for turbulent water (Jähne et al.,
302 1987).

303 3. Results

304 Physicochemical characteristics for each field campaign were categorized into the wet and dry seasons in accordance with
305 the precipitation records (Tables 2 and S1; Fig. S1). Water temperatures varied between 12 and 30 °C, with consistently

306 higher values in the wet than dry seasons (mean wet-dry difference = +5.9 °C). pH was lower in the wet than dry seasons
307 by ~0.35 units. TSM contents ranged between several to hundreds of mg L⁻¹ and were higher in the wet than dry seasons
308 by up to a factor of 40 (for LY04). The $\delta^{13}\text{C}_{\text{POC}}$ values ranged between -28 and -20‰ and were more negative in the dry
309 than wet seasons. Ammonium concentrations ranged between 0.3 and 16.5 μM and were higher in the wet than dry
310 seasons. In contrast, nitrate/nitrite and phosphate concentrations ranged from 1.7 to 48.3 μM and 0.01 to 0.31 μM ,
311 respectively. Neither of them differed significantly between seasons (linear mixed-effects model, $p > 0.05$; Table S1). Chl
312 *a* concentrations ranged from lower than the detection limit to 2.1 mg m⁻³ and were higher in the dry season than in the
313 wet season (except for DL). DIC concentrations and their $\delta^{13}\text{C}$ values varied between 1.26 and 2.88 mM and between -
314 6.8 and +0.6‰, respectively with no significant difference between seasons (linear mixed-effects model, $p > 0.05$). Sulfate
315 concentrations ranged between 1.03 and 2.06 mM for most sites (except for MLL01 at 2.92–3.59 mM) and were higher
316 in the wet than dry seasons. Sodium and calcium concentrations were lower in the wet than dry seasons.

Table 2: Physicochemical characteristics among sites and seasons.

Tributary (altitude)	n (sample numb. for wet or dry season)	T ¹ (°C)	pH	TSM ¹ (mg L ⁻¹)	POC (mg m ⁻³)	δ ¹³ C- POC (‰)	DOC (μM)	DIC (mM)	δ ¹³ C-DIC (‰)	Chl a (mg m ⁻³)	TIN ² (μM)	NH ₄ ⁺ (μM)	PO ₄ ²⁻ (μM)	SO ₄ ²⁻ (mM)
		n= 4, 3	n= 4, 3	n= 2, 3	n= 2, 3	n= 2, 3	n= 4, 3	n= 3, 3	n= 4, 3	n= 3, 2	n= 4, 3	n= 3, 3	n= 4, 3	n= 3, 3
MLL01 (699 m)	Wet seasons	23.4 ± 2.8	8.4 ± 0.4	157 ± 113	2432 ± 1868	-24.3 ± 0.3	30.4 ± 12.96	1.92 ± 0.05	-1.8 ± 2.1	0.07 ± 0.06	10.0 ± 2.5	4.6 ± 4.3	0.04 ± 0.05	3.36 ± 0.21
	Dry seasons	17.3 ± 4.7	8.1 ± 0.5	11 ± 16	152 ± 32	-27.4 ± 0.3	20.63 ± 1.71	1.91 ± 0.34	-2.7 ± 1.3	0.44 ± 0.08	6.8 ± 6.2	0.6 ± 0.3	0.05 ± 0.04	3.02 ± 0.13
	Total	20.8 ± 4.7	8.2 ± 0.4	70 ± 98	1064 ± 1560	-26.1 ± 1.7	26.21 ± 10.59	1.92 ± 0.21	-2.2 ± 1.8	0.22 ± 0.21	8.7 ± 4.3	2.6 ± 3.5	0.05 ± 0.04	3.19 ± 0.24
CWL01 (375 m)	Wet seasons	26.1 ± 2.4	7.8 ± 0.1	27 ± 32	1477 ± 1381	-23.4 ± 2.7	31.22 ± 16.03	1.81 ± 0.48	-2.8 ± 1.3	0.58 ± 0.38	6.0 ± 0.8	4.9 ± 1.9	0.06 ± 0.04	1.53 ± 0.04
	Dry seasons	20.2 ± 6.8	8.5 ± 0.3	12 ± 18	236 ± 129	-26.8 ± 1.0	23.96 ± 3.30	2.18 ± 0.37	-2.1 ± 1.9	1.33 ± 1.02	5.2 ± 3.9	2.5 ± 0.6	0.04 ± 0.03	1.61 ± 0.04
	Total	23.1 ± 5.6	8.2 ± 0.4	18 ± 22	732 ± 973	-25.4 ± 2.4	28.11 ± 12.13	1.99 ± 0.43	-2.5 ± 1.5	0.83 ± 0.67	5.7 ± 2.3	3.7 ± 1.8	0.05 ± 0.03	1.57 ± 0.06
DL (382 m)	Wet seasons	26.6 ± 2.2	7.9 ± 0.6	4 ± 3	291 ± 24	-25.9 ± 0.2	35.39 ± 19.96	2.16 ± 0.05	-1.9 ± 0.9	1.14 ± 0.54	16.0 ± 4.2	6.4 ± 6.5	0.14 ± 0.02	1.08 ± 0.04
	Dry seasons	21.9 ± 7.3	8.4 ± 0.2	11 ± 18	160 ± 63	-26.4 ± 0.4	20.26 ± 3.40	2.43 ± 0.41	-1.9 ± 0.6	0.64 ± 0.24	18.0 ± 3.0	1.5 ± 1.3	0.15 ± 0.06	1.13 ± 0.12
	Total	24.6 ± 5.2	8.2 ± 0.5	8 ± 13	212 ± 86	-26.2 ± 0.4	28.9 ± 16.38	2.29 ± 0.30	-1.9 ± 0.7	0.94 ± 0.48	16.8 ± 3.6	4.3 ± 5.4	0.14 ± 0.04	1.11 ± 0.08
LY04 (238 m)	Wet seasons	27.4 ± 2.3	8.0 ± 0.2	499 ± 172	9361 ± 4358	-20.4 ± 0.3	22.97 ± 4.02	2.30 ± 0.38	-2.9 ± 1.4	0.05 ± 0.09	9.6 ± 4.5	7.1 ± 3.2	0.08 ± 0.03	1.70 ± 0.24
	Dry seasons	21.1 ± 6.0	8.4 ± 0.3	15 ± 15	174 ± 48	-27.5 ± 0.4	17.42 ± 3.94	2.34 ± 0.43	-3.9 ± 1.0	0.37 ± 0.09	8.3 ± 2.8	2.2 ± 0.7	0.07 ± 0.00	1.41 ± 0.06
	Total	24.7 ± 5.1	8.2 ± 0.3	208 ± 280	3849 ± 5484	-24.7 ± 3.9	20.59 ± 4.7	2.41 ± 0.32	-3.3 ± 1.2	0.18 ± 0.19	9.0 ± 3.6	4.7 ± 3.4	0.07 ± 0.02	1.56 ± 0.22
BNE (7 m)	Wet seasons	28.0 ± 2.6	7.8 ± 0.1	106 ± 131	2282 ± 2718	-25.0 ± 1.4	45.13 ± 22.48	2.58 ± 0.45	-4.9 ± 2.5	0.78 ± 1.03	31.0 ± 14.1	7.3 ± 6.2	0.19 ± 0.09	1.84 ± 0.20
	Dry seasons	21.5 ± 6.8	8.4 ± 0.3	12 ± 17	245 ± 73	-26.4 ± 0.6	35.62 ± 10.05	2.02 ± 0.28	-5.7 ± 0.2	1.73 ± 0.43	30.1 ± 10.5	1.7 ± 0.2	0.25 ± 0.07	1.46 ± 0.09
	Total	25.2 ± 5.5	8.4 ± 0.2	49 ± 84	1060 ± 1759	-25.8 ± 1.1	41.05 ± 17.67	2.30 ± 0.45	-5.2 ± 1.8	1.16 ± 0.92	30.6 ± 11.7	4.9 ± 5.3	0.21 ± 0.08	1.65 ± 0.25

* Parameters shown in bold are significantly different between two seasons (Wilcoxon test, *p* < 0.05). Detailed data are provided in Table S1.

¹ T: water temperature; TSM: total suspended matter.

² TIN includes nitrate and nitrite (nitrite was below detection limit).

323 Uptake rates for ^{13}C -labeled DIC under light conditions varied from 0.03 to 1.98 $\text{mg C m}^{-3} \text{ hr}^{-1}$ (Fig. 3a; Table S2). The
324 rates measured in August were either significantly higher than (for MLL01, LY04, and BNE) or comparable (for CWL01
325 and DL) with those in other months (within-site Kruskal-Wallis, $p < 0.05$; Fig. 3a). The rates were also higher for BNE
326 than for MLL01, CWL01, and DL (Kruskal-Wallis, $H=18.15$, $p = 0.001$; Dunn's post-hoc, $p < 0.05$; Fig.3a). Within-site
327 temporal variation was significant at all five sites (Kruskal-Wallis, $p < 0.05$; Fig. 3a). In contrast, incubations with ^{13}C -
328 labeled DIC under dark conditions exhibited a drastically different pattern (Fig. 3b). Most rates were either below the
329 detection limit or less than 0.05 $\text{mg C m}^{-3} \text{ hr}^{-1}$. Exceptions occurred for sites MLL01 (0.7 $\text{mg C m}^{-3} \text{ hr}^{-1}$), LY04 (1.6 mg
330 $\text{C m}^{-3} \text{ hr}^{-1}$), and BNE (0.19 $\text{mg C m}^{-3} \text{ hr}^{-1}$) in August.
331

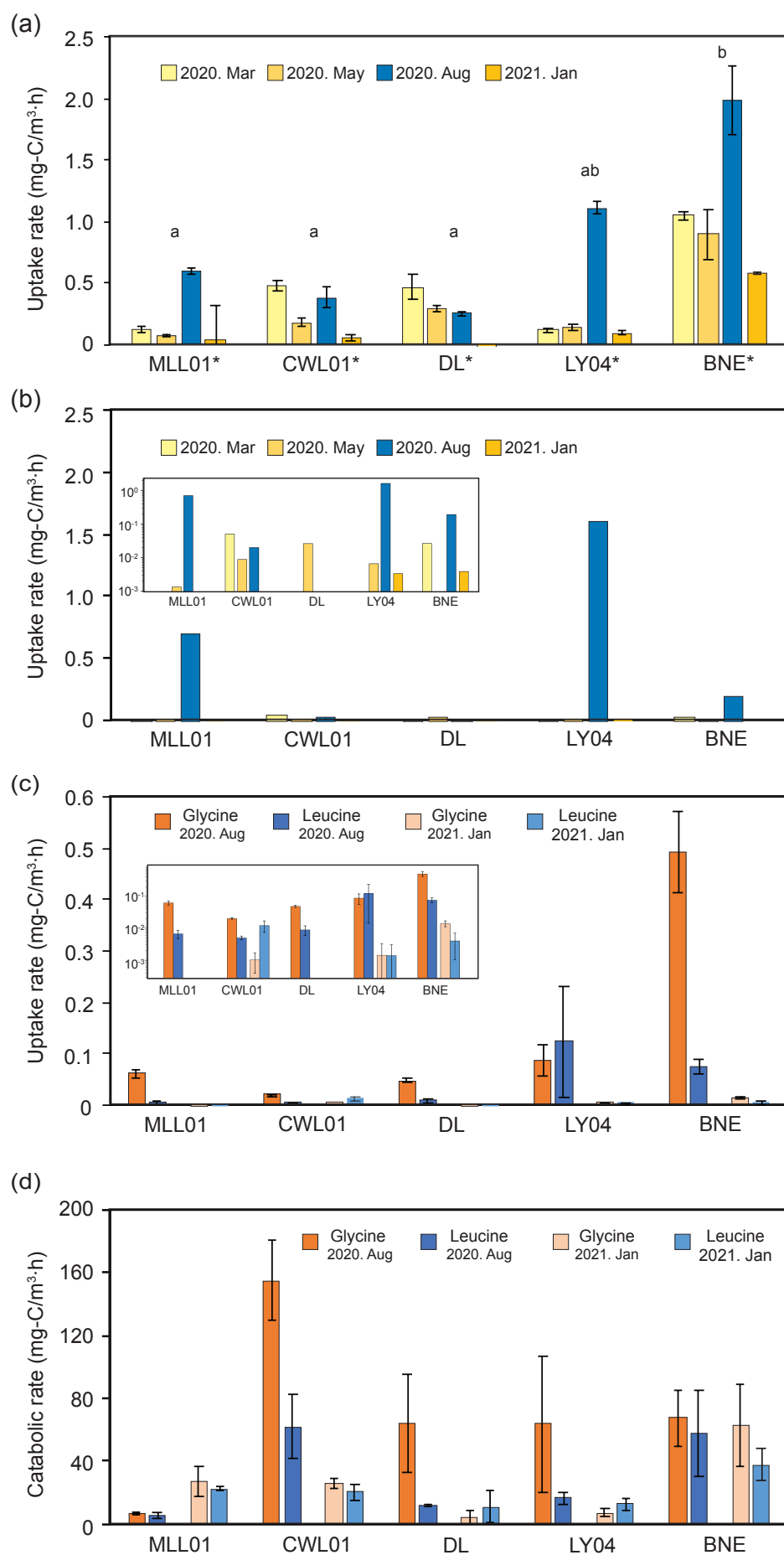


Figure 3: Carbon transformation rates along the Beinan River from upstream (MLL01) to downstream (BNE). DIC uptake rates under (a) light and (b) dark incubation conditions across four sampling dates representing two seasons: wet season (August 2020) and dry season (March 2020, May 2020, and January 2021). The inset in panel (b) shows the same data on a logarithmic scale. (c) Amino acid uptake rates and (d) catabolic rates for glycine and leucine measured during the wet season and dry season. The inset in panel (c) shows the same data on a logarithmic scale. Error bars represent standard deviation of triplicate measurements (n=3) for the light DIC, glycine, and leucine treatments. The dark DIC condition was a single incubation (n=1) and is shown without error bars. Missing values indicate measurements below zero. Different letters above bars indicate statistically significant differences among sites based on Kruskal-Wallis test (panel a: $H=18.15, p=0.001$) followed by Dunn's pairwise post-hoc test. Note that panel (c) did not reach overall significance ($p>0.05$) but pairwise comparisons identified BNE as distinct from other sites. No significant among-sites differences were detected for catabolic rates in panel (d) ($H=5.70, p=0.228$). Asterisks following site names in panel (a) indicate significant within-site temporal variation (Kruskal-Wallis, $p < 0.05$).

Uptake rates of incubations with ^{13}C -labeled amino acids ranged from lower than the detection limit to $0.49 \text{ mg C m}^{-3} \text{ hr}^{-1}$ (Fig. 3c; Table S2). These rates tended to be higher in August than in January at all sites. Uptake rates with glycine were generally higher than those with leucine with the exception for LY04 in August (Fig. 3c). At the among-site level, BNE was distinct from the other sites for glycine uptake (Kruskal-Wallis pairwise comparisons, $p < 0.05$). For comparison, catabolic rates ranged from 4.5 to $154.4 \text{ mg C m}^{-3} \text{ hr}^{-1}$ and varied spatially and temporally (Fig. 3d; Table S2). Catabolic rates with glycine were also higher than those with leucine across most incubations, with the exceptions of DL and LY04 in January (Fig. 3d). The catabolic rates appeared to be higher in August than in January for sites CWL01, DL, and LY04, but were comparable between the two months for site BNE. No significant among-site differences were detected (Kruskal-Wallis, $H=5.70, p=0.23$). Within-site difference between two time points was not tested, because a two-sample rank test with only three replicates per group ($n=3$) cannot yield a statistically significant result. By contrast, the catabolic rates for MLL01 were higher in January than in August. Most of the added amino acids were utilized for respiration (as the product of total CO_2 ; $>99\%$) rather than biosynthesis.

To identify the environmental drivers of the metabolic rates, Pearson correlation analyses were performed between the rates and the measured environmental parameters (Fig. S4). DIC uptake rates under light conditions were correlated significantly ($p < 0.01$) with multiple parameters, including DOC, POC, $\delta^{13}\text{C}$ -POC, nitrate/nitrite, phosphate, TSM, water temperature, and NH_4^+ . The phototrophic rates constrained by the difference between rates under light and dark were correlated with DOC, nitrate/nitrite, and phosphate. In contrast, DIC uptake rates under dark conditions were correlated most strongly with sediment-associated parameters (TSM, POC, $\delta^{13}\text{C}$ -POC, and NH_4^+). Glycine uptake rates were correlated with DOC, nitrate/nitrite, and phosphate, whereas leucine uptake was correlated with TSM, POC, $\delta^{13}\text{C}$ -POC, and NH_4^+ . Glycine catabolic rates showed a strong negative correlation with DOC concentration, while leucine catabolic rates showed no significant correlation with any environmental parameter.

Scaling measured rates to the catchment level yielded net metabolic CO_2 production fluxes of $5.16 \text{ mol m}^{-2} \text{ yr}^{-1}$ for MLL01, $21.11 \text{ mol m}^{-2} \text{ yr}^{-1}$ for CWL01, $4.22 \text{ mol m}^{-2} \text{ yr}^{-1}$ for DL, $5.35 \text{ mol m}^{-2} \text{ yr}^{-1}$ for LY04, and $24.44 \text{ mol m}^{-2} \text{ yr}^{-1}$ for BNE (Table S5). The total yield of planktonic metabolic CO_2 for individual sites ranged from 10^5 to 10^7 mol yr^{-1} , with a catchment-wide total of $7.89 \times 10^7 \text{ mol yr}^{-1}$. For comparison, CO_2 evasion fluxes through river-air exchange ranged from 0.20 – $1.76 \text{ mol m}^{-2} \text{ d}^{-1}$ for CWL01, 3.97 – $17.3 \text{ mol m}^{-2} \text{ d}^{-1}$ for MLL01, 0.27 – $0.73 \text{ mol m}^{-2} \text{ d}^{-1}$ for DL, 0.39 – $1.56 \text{ mol m}^{-2} \text{ d}^{-1}$ for LY04, and 0.20 – $0.90 \text{ mol m}^{-2} \text{ d}^{-1}$ for BNE (Table S6). Summing exchange rates across the catchment yielded a total annual emission of approximately $2.6 \times 10^9 \text{ mol yr}^{-1}$. Planktonic microbial processes contributed approximately 2–4% of the total annual CO_2 evasion rate (average at 3.8%; Table S5 and S6).

Bacterial and archaeal 16S rRNA gene abundances for river water ranged from 2.3×10^3 to $3.9 \times 10^7 \text{ copies} \cdot \text{L}^{-1}$ and from 5.6×10^4 to $5.5 \times 10^6 \text{ copies} \cdot \text{L}^{-1}$, respectively (Fig. S3). The abundances of *cbbL* ranged from 1.8×10^3 to $7.2 \times 10^5 \text{ copies}$

377 L⁻¹. All the gene abundances were generally higher in January than those in other months (mostly by more than one order
 378 of magnitude) except at BNE. The variation in gene abundance between January and August was most pronounced at
 379 LY04, where bacterial 16S rRNA gene copy numbers were ~2 orders of magnitude smaller in August than in January
 380 (Fig. S3). By comparison, archaeal 16S rRNA gene abundances at BNE were relatively stable across the four sampling
 381 months. Statistical examinations on season were not conducted owing to the absence of biological replication for the
 382 gene-abundance measurement.

383 A total of 532,787 reads of 16S rRNA gene amplicons were obtained and classified into 13,005 ASVs. The observed
 384 ASVs and Chao1 based on the rarefied dataset (n=5220) ranged between 200 and 1600 and between 150 and 2300,
 385 respectively (Table S3). The Shannon index spanned from 4.4 to 6.9. Alpha diversity varied among sites and sampling
 386 intervals (Fig. 4a). Kruskal-Wallis tests did not detect statistically significant difference among sites (Observed ASVs: H
 387 = 6.99, *p* = 0.14; Chao1: H = 6.35, *p* = 0.17; Shannon: H = 8.36, *p* = 0.08) or between seasons (all *p* > 0.72). Nonetheless,
 388 Shannon diversity tended to be highest at the upstream tributary CWL01 (mean = 6.4) and lowest at the river mouth BNE
 389 (mean = 5.0). At MLL01 and LY04, observed ASVs and Chao1 dropped markedly during the early wet season (July),
 390 whereas CWL01, DL, and BNE showed more stable values across the study period.

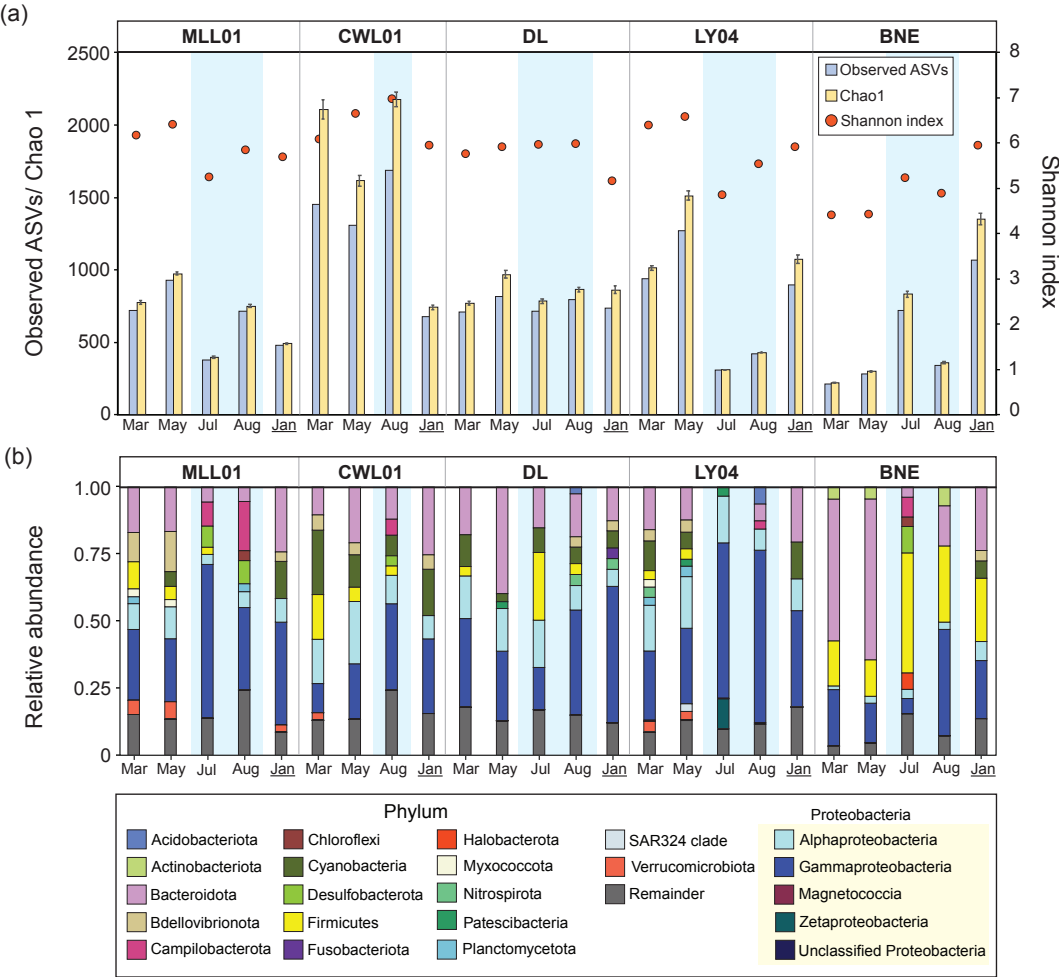


Figure 4: (a) Alpha diversity indices (observed ASVs, Chao1, Shannon index) and (b) community compositions at the phylum level, with Proteobacteria resolved at the class level. Error bars on Chao1 represent ± 1 SE estimated from rarefaction. Time intervals with blue background represent the wet season.

Community compositions varied temporally and spatially to different degrees with the major phyla including Bacteroidota, Cyanobacteria, Firmicutes, Campilobacterota and Desulfobacterota, Proteobacteria (Alpha and Gamma

divisions) (2–20%; Fig. 4b). Gamma-Proteobacteria appeared to be more abundant in most time intervals and at most sites except for BNE. Cyanobacteria was more abundant in the dry season than in the wet season, and vice versa for Campilobacterota and Desulfobacterota (Fig.4b). For BNE, Bacteroidota constituted the major phylum for two out of three dry season samples. Firmicutes combined with Gamma-Proteobacteria outnumbered (>54%) the others during the wet season and January. Among all detected ASVs, ASVs with sequences related to Thiotrichaceae, Erysipelotrichaceae, and Phormidiaceae represented the most abundant ones. However, their abundances varied dramatically among sites.

Beta diversity analyses showed that community composition was structured primarily by site (PERMANOVA: $F_{4,19} = 2.01$, $R^2 = 0.30$, $p = 0.001$), while the seasonal effect was marginal ($F_{1,22} = 1.29$, $R^2 = 0.06$, $p = 0.087$). The joint site and season model explained 35% of variance ($p = 0.001$). In the CCA ordination (Fig. 5), BNE samples deviated from the cluster formed by the other sites and were associated with elevated DOC. For the remaining sites, dry-season samples clustered while wet-season samples were more dispersed and correlated positively with TSM, POC, and ^{13}C -POC. All four environmental vectors were significant (envfit, $p < 0.05$; $r^2=0.62$ – 0.96 ; Fig. 5).

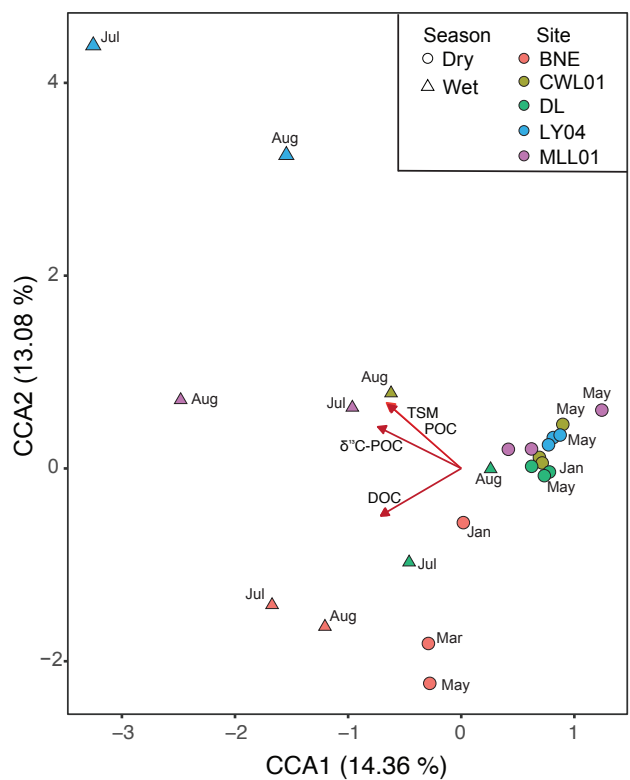


Figure 5. Canonical correspondence analysis (CCA) ordination of microbial community composition of 16S rRNA gene amplicons (ASV proportion). Significant environmental vectors (envfit, 9999 permutations, $p < 0.05$) are shown as solid arrows: TSM ($r^2=0.94$, $p=0.006$), POC ($r^2=0.96$, $p=0.007$), DOC ($r^2=0.62$, $p=0.011$), and $\delta^{13}\text{C}$ -POC ($r^2=0.65$, $p=0.035$). Symbols indicate site (color) and season (circle = dry, triangle = wet).

4. Discussion

4.1 Solute and nutrient status, and river metabolism

The general physicochemical characteristics of river water (pH, major ions, TOC, TSM, and ^{13}C -DIC) were comparable with the data reported previously (Wang et al., 2024). These solute characteristics are mostly derived from carbonate dissolution (>80% of the total solute loads) driven by sulfuric acid produced by microbially mediated pyrite oxidation

(Wang et al., 2024). Considering that a great portion of discharge is attributed to monsoon or typhoon, solute yields from carbonate dissolution during these extreme events dominate over the quiescent period and could be translated to a significant flux of CO₂ emission that constitutes a disproportionally large fraction of global flux (~1% from 0.002% of three major catchments in Taiwan). The high contribution pattern of carbonate weathering to the solute budget has also been observed for other active orogens (e.g., New Zealand and Himalayas) (Emberson et al., 2016; Kemeny et al., 2021). Furthermore, high water regime during summer monsoon or typhoons drains erodible materials (sediments accumulated in banks or hillslopes) into channels. As a result, TSM concentrations increased to a range of hundreds of mg L⁻¹ by up to a factor of 40 (at LY04) between the wet and dry seasons (Table 2). This magnitude is far less than previous observations during the extreme events (up to 10 g L⁻¹; Dadson et al. 2003; Kao and Milliman 2008; Wang et al., 2024), given that the annual precipitation during the sampling period was much less than previous years (1856 mm for this study period versus ten-year average of 3402 mm for the most upstream Siangyang weather station; CWB annual report), but could still be sufficient to influence the expression of investigated metabolisms (see the next section). Similar increases in POC concentrations were also observed (Table 2). For comparison, dissolved cation concentrations were characterized by various degrees of dilution caused by the increase in discharge (linear mixed-effects model, $p < 0.05$; Table S1). In contrast, sulfate is significantly more abundant in the wet than dry seasons (linear mixed-effects model, $p = 0.004$), a pattern consistent with enhanced pyrite oxidation during sediment-mobilization in this catchment (Wang et al., 2024) and the elevated wet-season abundance of sulfur-oxidizing taxa such as *Thiobacillus* and *Sulfuricurvum* (Section 4.6).

While nitrate/nitrite and phosphate concentrations did not differ significantly between seasons, Chl *a* concentrations were significantly higher in the dry season and ammonium concentrations were significantly higher in the wet season (linear mixed-effects model, both $p < 0.05$; Table 2). As ammonium represents the degradative product of organic particulates, the higher ammonium concentrations in the wet season suggest either higher organic degradation along river transit or the purging of soil porewater more extensively during high water regime (Calmels et al., 2011). It is also worth noting that the comparable nitrate/nitrite and phosphate concentrations between seasons or higher ammonium concentrations in the wet season could be translated into a higher yield of these nutrients given a much higher discharge. The decomposition of suspended particulate organic matter can release ammonium into river water. Part of ammonium production may have been even channeled from the degradation of amino acids, which can be also sourced to the degradation of suspended organic particulates. As indicated by our results, amino acid mineralization occurred across all investigated sites distributed from the upstream to estuary. Their rates were also higher in the wet than dry seasons, coinciding with the increase input of TSM. However, our results are not sufficient to further address whether the enhanced amino acid rates in the wet season can be tracked to the increase supply of amino acids from the degradation of organic particulates. Alternatively, soil development is limited in the investigated catchment. High erosion and heavy precipitation associated with extreme hydrologic events could have drained the stagnant soil porewater along with soil particulates and groundwater residing in fracture network rapidly into the rivers. As these water bodies preserve the degradative products of organic matters and their transport is slow (Alley et al., 2002; Calmels et al., 2011), these nutrient constituents would be accumulated to a high level during the quiescent period and only be rapidly purged when the erosion and high hydraulic head are imposed. As ammonium concentrations tended to increase from the upstream headwater (MLL01: 2.6 μM) toward the river mouth (BNE: 4.9 μM) (3.7–4.7 μM; Table 2), the concentration gradient was even more pronounced during the wet season than the dry season. The data pattern seems consistent with progressive accumulation of soil-derived inputs along the catchment under high flow conditions. Finally, water temperatures during the wet season were systematically ~6°C higher than during the dry season (Table 2). Enhanced microbial degradation of suspended or soil organic matter driven by temperature increase may lead to the increased ammonium release into the river water. Overall,

current data does not allow us to identify the main cause driving enhanced ammonium concentrations in the wet season. High frequency monitoring of river water and groundwater across the event period is warranted to gauge the mechanism of nutrient release from the soil. Such a data pattern is also comparable with nutrient-deprived oligotrophic state commonly observed for open ocean (Overholt et al., 2022).

Chl *a* concentrations were significantly higher during the dry season than during the wet season (linear mixed-effects model, $p = 0.007$), by a factor of at least 2.2 (except for site DL). Pearson correlation analysis revealed a significant negative correlation between Chl *a* and TSM ($r = -0.46$, $p < 0.05$) and no significant correlation between Chl *a* and POC ($r = -0.41$, $p = 0.06$) (Table S4). Chl *a* concentrations at sites MLL01 and LY04 during the wet season were barely detectable. While nutrient concentrations (nitrate/nitrite and phosphate) do not vary considerably between sites, nutrients do not appear to be a deterministic factor in controlling phototrophic activity. Instead, the load of suspended sediment can affect the expression of phototrophy even at a scale of hundreds of mg L^{-1} , a level that is not particularly unusual for mountainous rivers. Potential mechanisms inhibiting phototrophic activity include the shading created by dense sediments, grain pulverization, and abrasion (Stanley et al., 2010). The results are consistent with O'Donnell and Hotchkiss (2022), who found that the primary production and ecosystem respiration impacted by high flow events deviated from the baseline represented by the mean daily metabolism based on continuous dissolved oxygen concentrations. Their results also indicate that both GPP and ER rates were reduced by flash flow with GPP rates varying at a greater magnitude than ER (Blaszczak et al., 2019; Hall et al., 2015).

4.2 Autotrophic carbon fixation: rates and controlling factors

DIC uptake rates were significantly correlated with TSM, POC, $\delta^{13}\text{C}$ -POC, and NH_4^+ regardless of light availability (Fig. S4). Under light conditions, DIC uptake rates were also correlated with temperature, DOC, and nutrients. DIC rates for the wet season could be qualitatively categorized into two patterns. The first category is represented by sites CWL01, DL, and BNE where rates with light were higher than those without light (Fig. 3a and 3b). The largest difference at $43 \text{ mg C m}^{-3} \text{ d}^{-1}$ was recovered from the most downstream site BNE (Table S2). The second category is represented by sites MLL01 and LY04 where rates with light were either comparable with or slightly less than those without light. For incubations with DIC for the dry season, rates with light were generally much larger than those without light. In fact, rates without light were below the detection for sites MLL01, DL, and BNE. The only exception occurs for site CWL01 where DIC uptake rates with light were slightly higher than those without light. The results in part corroborate the Chl *a* pattern but offer an additional dimension for constraining river metabolism.

The contrast pattern described above is apparently consistent with light limitation imposed by suspended sediment, which was far higher at MLL01 and LY04 (wet-season TSM ~ 157 and $\sim 499 \text{ mg L}^{-1}$) than at CWL01, DL, and BNE ($\sim 4 - 106 \text{ mg L}^{-1}$). Furthermore, differences in rates with and without light could be interpreted as the phototrophic rates assuming that lithoautotrophic rates (obtained from dark conditions) are constant regardless of whether light is available. In this regard, phototrophic rates for sites MLL01 and LY04 were lower in the wet season and even completely inhibited with the replacement of lithoautotrophy. In contrast, phototrophic rates were greater in the wet season than in the dry season for the other three sites even though lithoautotrophy was substantially reduced during the dry season. Additionally, the significantly positive correlations between DIC rates under light conditions and TSM, POC, and $\delta^{13}\text{C}$ -POC contradict with the expression of phototrophy. This pattern could be largely accounted for by the fact that a large fraction of the DIC rates with light (115-145%) were attributed to the lithoautotrophic rates during the wet season (Table S2). Therefore, the significant correlation for light conditions resembles that for dark conditions. By comparison, the significant correlation between the difference in DIC rate and nutrient concentration is compatible with the fact that the primary production is

highly dependent on the nutrient availability in aquatic environments (Cloern, 1999; Domingues et al., 2005). As the nutrients generated from the degradation of organic matters accumulate along the river flow, BNE was characterized by the highest concentrations of nutrients and phototrophic rates in the wet season (Fig. 3). Notably, the Chl *a* concentration did not show a significant correlation to the phototrophic rate (Fig. S3).

4.3 Particulate-mediated lithoautotrophy in the wet season

The incubation with DIC under dark conditions yielded DIC uptake rates spanning from lower than the detection to 1.6 mg C m⁻³ hr⁻¹ (Fig. 3; Table S2). These rates constitute mostly a small fraction (<5%) of the rates under light conditions but become generally high and even comparable with the rates under light conditions for samples collected during the period of high sediment load (LY04 and MLL01 in August). As these two sub-catchments are susceptible to high erosion of slate formations, such a spatial pattern of dark DIC uptake is consistent with the control of geology rather than the upstream-downstream position along the catchment. The rate pattern is intriguing and suggests the intimate relationships with the potential substrates supplied from the suspended particulates or reduced ions from the groundwater influx. For particulate matters, potential substrates include reduced sulfur or iron-bearing minerals (e.g., pyrite) and ammonium encapsulated within the interlayer of clay minerals (Petit et al., 2006). Pyrite is prevalently distributed in meta-sedimentary rocks of the catchment and has been identified as the main solute and CO₂ contributor of weathering (Blattmann et al., 2019; Bufe et al., 2021). A previous study conducted on the same catchment has shown a highly positive correlation of the yields between riverine sulfate, suspended sediment, and sulfur oxidizer (mainly composed of *Thiobacillus* and *Sulfuricurvum* members) (Wang et al., 2024). The data pattern suggests that sulfate/sulfuric acid is primarily generated by the microbially mediated oxidation of pyrite in erodible materials in riparian zones or hillslopes. As the torrential precipitation proceeds, these materials along with the sulfur oxidizers are drained into the river channels and recovered by sampling and analysis. The data corroborate with the fact that erosion of bedrocks and the following pulverization on hillslopes or in river channels facilitate the exposure of pyrite to the atmosphere or air-infiltrated pore space, thereby rendering the oxidative reaction that produces sulfuric acid for dissolution of carbonate or other minerals (Hilton and West, 2020). Furthermore, the particulate pulverization and abrasion can also disrupt the cleavage or interlayer structure of clay minerals, thereby releasing ammonium into the surrounding (Yu et al., 2023). While river water is presumably saturated with the atmospheric oxygen, pyrite and ammonium associated with suspended particulates are readily available for lithoautotrophic microbial exploitation and colonization. For groundwater influx for river, potential substrates for lithoautotrophy include ferrous iron, ammonium, and sulfide, all of which could be produced by either microbial reduction of oxidants (e.g., iron oxyhydroxide, nitrate, and sulfate) or degradation of organic matters (for the release of ammonium). Considering that sulfate minerals have not been identified or recovered from the catchment to date, ferrous iron and ammonium produced in subsurface aquifers could be drained and discharged into rivers. Indeed, ammonium concentrations were significantly higher for the wet than for the dry seasons (Table 2). Our current analyses also revealed that potential sulfur oxidizers related to these two genera (*Thiobacillus* and *Sulfuricurvum* members) and others (e.g., *Sulfurovum*, *Sulfurifustis*) and ammonium oxidizers related to *Nitrosomonas* and *Nitrosopumilus* constituted a significant proportion of communities (>10%) in the wet season as compared to 5% in the dry season. Detailed experiment design is required to pinpoint the exact substrate involved in the aquatic lithoautotrophy.

4.4 Heterotrophic amino acid metabolism and controlling factors

In contrast to autotrophic carbon fixation, heterotrophic metabolism showed distinct seasonal patterns and substrate preferences. Glycine and leucine uptake rates were correlated with nutrients and particulate characteristics (concentration and isotopic composition), respectively (Fig. S4). As the nutrients represent the degradative product of organic

543 particulates, these relationships seem to suggest that the direct uptake of amino acids by riverine community members is
544 linked to the metabolites generated by particulate degradation (for glycine) or particulate-associated populations (for
545 leucine). For the former scenario, metabolites or nutrients released from the particulate degradation accumulated along
546 the flow, peaking at the estuary site (BNE) when compared with upstream sites. Therefore, the correlation relationships
547 suggest that enhanced abundances of nutrients facilitate to boost the activity of a riverine population preferentially for
548 glycine uptake. Such a relationship is also analogous to the priming effect on soil microbiota where the addition of
549 exogenous substrate or substance promotes further degradation of organic matters (Blagodatskaya and Kuzyakov 2008;
550 Chen et al., 2014). On the other hand, the latter scenario suggests that the leucine uptake is more favorable for upstream
551 sites where TSM concentrations were higher. The correlation also points to an intimate relationship with the size of the
552 population attached to particulate organic matters. With a larger influx of TSM in the wet season, the size of the attached
553 population could have been correspondingly enhanced, providing an amplified body of microorganisms with functional
554 preferences on leucine uptake. The inference also suggests a divergent modulation of physiological preferences on glycine
555 and leucine uptakes mediated by two distinct populations. The inference may be better constrained by expanding the
556 incubations to various concentrations of particulates and simultaneous analyses of stable isotope probing of cellular
557 components (proteins, lipids, or nucleic acids) to identify the plausible populations actively incorporating either of these
558 amino acids.

559 The exploitability of organic substrates for heterotrophy is worth noting. Glycine uptake rates were generally higher than
560 leucine uptake rates for the wet season (except for LY04, where both rates were comparable) (Fig. 3c). In the dry season,
561 these two uptake rates were comparable but noticeably much lower than those in the wet season. For comparison, glycine
562 catabolic rates were higher than leucine catabolic rates for CWL01, DL, and LY04 in the wet season. Both rates were
563 comparable to each other at other sites (Fig. 3d). Overall, the selectivity of glycine over leucine for the wet season was
564 prevalent (at four out of five sites for uptake and three out of five sites for catabolism). The cause of substrate selectivity
565 may be attributed to the preferential utilization of simply structured substrate by specific community members or
566 enzymatic expression (Geisseler and Horwath 2014; Palenik and Morel 1990). The selectivity for glycine likely reflects
567 higher metabolic efficiency of glycine-centered pathways. The reductive glycine pathway (rGlyP) and glycine cleavage
568 system (GCS) offer highly ATP-efficient routes for both biosynthesis and energy generation (Dronsella et al., 2025;
569 Kikuchi et al., 2008), providing a more consistent mechanistic basis for glycine metabolism across seasons. In contrast,
570 leucine catabolism requires a complex multi-step pathway involving at least six enzymatic reactions to yield acetyl-CoA
571 and acetoacetate (Brosnan and Brosnan, 2006; Massey et al., 1976), making respiratory oxidation costly. Therefore,
572 microorganisms would preferentially incorporate leucine into proteins (Kirchman, 2001; Kirchman et al., 1985), such that
573 leucine-derived rates provide a conservative estimate for heterotrophic activity. As both amino acids are required for
574 biosynthesis, further experiments are needed to clarify the expression of specific metabolic pathways.

575 The results for heterotrophic incubations in this study revealed that catabolic rates exceeded assimilation rates by at least
576 two orders of magnitude (Fig. S5). The great rate contrast was partially accounted for by the fact that ^{13}C was labeled at
577 the carboxyl position of amino acids in our incubations. The carboxyl carbon in amino acids would be primarily cleaved
578 and converted into CO_2 , whereas all the other unlabeled carbon would be assimilated and partially converted into CO_2 at
579 the cost of energy expenditure (Hill et al., 2013; Suttle et al., 1991). Therefore, carbon uptake would be primarily sourced
580 to non-carboxyl carbon with limited contribution from carboxyl carbon. Other than the site preference associated with
581 labeled position which would result in the underestimates of both rates, the ratios of catabolic to uptake rates are still
582 related to community activity and composition modulated by environmental parameters. Except for January incubation
583 with leucine for CWL01, all the other incubations with amino acids yielded a ratio of catabolic to uptake rates for January

greater than for August. The ratios even reached an infinite level (uptake rate below the detection) for MLL01 and DL in January. The overwhelmingly large ratios were primarily controlled by heterotrophic assimilation at extremely low levels during the dry season when sediment supply was low. Although the incubations were provided with readily available amino acids, the slow assimilation suggests that the communities in the dry season may be customized to a deprived supply of nutrients and small organic molecules generated from the degradation of particulate organic matter. Therefore, any solid or soluble substrate available for microbial utilization would be initially diverted to catabolism for energy generation to sustain certain maintenance activities (McInerney et al., 2010). Thus, even when the amino acids were provided during incubations, their assimilation was not immediately enhanced under such energy-limited conditions for heterotrophs. Conversely, communities in the wet season were accessible to the abundant supply of particulate organic matters to catalyze metabolisms for energy generation or assimilation. Consequently, a relative larger fraction of the fed amino acids was diverted to assimilation or biosynthesis (Table S2, Fig. S5). Nevertheless, the heterotrophy in such oligotrophic mountainous rivers yields excessive CO₂ that is susceptible to the net emission through the air-water exchange.

4.5 Implications for river heterotrophy and carbon cycling

The rate comparison between autotrophy and heterotrophy (assimilation plus catabolism) indicates that the planktonic compartment of the Beinan River is net heterotrophic, producing excess CO₂ through planktonic microbial processes. As the incubations were performed over a short period of time (4–6 hrs), the physiological status of community members likely resembles *in situ* conditions. For DIC-based measurements provided with ¹³C at a concentration less than 5% of the ambient DIC pool, the derived rates were considered to bracket the range of true rates. For amino acid-based measurements, the 50 μM addition of amino acid exceeds the ambient concentration and half-saturation constants (K_m) for oligotrophic systems (11 – 79 nM in Boysen et al., 2022; 1.41 μM in Brailsford et al., 2019). Therefore, our experimental setup may represent the possible stimulation of uptake and respiration for our river systems. Furthermore, part of unlabeled carbon in amino acids assimilated and eventually respired were not quantified in our experiments, the obtained rates could be mostly derived from the carboxyl ¹³C in amended amino acids. Therefore, our measurements represent an underestimate of respiration under the experimental setup. To provide a first order and conservative quantification on the possible heterotrophic CO₂ production, the leucine catabolic rates were arbitrarily chosen to estimate the carbon export at the catchment scale.

We further note that the experimental design captures only part of metabolisms on dissolved organic substrates and does not directly probe the decomposition of pre-existing suspended POC. It is also worth noting that the current estimates constrain only the planktonic compartment of river metabolisms. The benthic and hyporheic zones, which are connected to the river water and contribute to the cycling of organic carbon, were not measured in this study. The current results should not be interpreted as an estimate of total river metabolism.

Our planktonic metabolic CO₂ production (7.89×10^7 mol yr⁻¹) accounts for less than 5% of both river-atmosphere CO₂ exchange flux ($\sim 2.6 \times 10^9$ mol yr⁻¹) and CO₂ production from chemical weathering (2.8×10^9 mol yr⁻¹; Wang et al., 2024) and petrogenic carbon oxidation (1.7 - 2.9×10^9 mol yr⁻¹; Lien et al., 2025). This pattern indicates that net CO₂ emissions through the river-air interface are overwhelmingly driven by the weathering of minerals and petrogenic carbon. While benthic and hyporheic contributions may have been reduced during the high-water period (Battin et al. 2023; Hall et al., 2015), they are likely substantial during dry-season low-TSM conditions when the water column is clear and light can reach the stream bed. To place the planktonic contribution in the broader context of stream metabolism, our rates were

623 compared with rates reported for morphologically comparable upland streams. Our measured DIC uptake rates (0.03-1.98
624 mg C m⁻³ h⁻¹) were transformed into a flux of 4×10^{-4} to 2×10^{-2} g C m⁻² d⁻¹ assuming an average water depth of 0.5 m.
625 By comparison, benthic gross primary production fluxes for clear upland streams typically range from $\sim 10^{-1}$ to several g
626 C m⁻² d⁻¹ (Hall et al., 2015; Bernhardt et al., 2022). Therefore, planktonic autotrophic fluxes for the Beinan River are one
627 to two orders of magnitude lower than benthic primary production fluxes reported elsewhere. In contrast, planktonic
628 heterotrophic catabolic fluxes (0.055-1.85 g C m⁻² d⁻¹) for the Beinan catchment fall within the typical range of total
629 ecosystem respiration fluxes reported for upland streams (~ 0.5 to several g C m⁻² d⁻¹; Battin et al., 2008; Hall et al., 2015;
630 Bernhardt et al., 2022). Because ecosystem respiration in those studies encompasses planktonic, benthic, and hyporheic
631 contributions rather than separating them, our planktonic-only rates suggest that the planktonic compartment can be a
632 significant fraction of total stream respiration. More detailed measurements are warranted to validate whether different
633 compartments of the Beinan river systems contribute to the net carbon export in a pattern resembling other similar
634 catchments.

635 Previous studies based on dissolved oxygen data demonstrate that both fluxes of net ecosystem production (NEP) and
636 CO₂ emissions derived from river-air exchange vary over several orders of magnitude as the discharge or stream order
637 increases for the catchments distributed over contiguous US and European boreal regions (Dodds et al., 2013; Cory et al.,
638 2014; Hotchkiss et al., 2015; Rasilo et al., 2017; Lupon et al., 2019; Bernal et al., 2022). Using a discharge-weighted
639 regression, Hotchkiss et al. (2015) found that NEP fluxes varied much less. Such a regional data pattern also suggests that
640 the contribution fraction of NEP to the total emission increases from $\sim 15\%$ to $\sim 50\%$ as the discharge increases to hundreds
641 of cms (cubic meter per second). Only limited data simultaneously covering NEP, CO₂ emissions, and discharge are
642 available for individual catchments. These studies show that the fractions range from 17% to 75% for rivers in Canada
643 (Rasilo et al., 2017), from 17% to 51% in Sweden (Lupon et al., 2019), $\sim 22\%$ in southeastern US (Dodds et al., 2013),
644 and from 51% to 57% in northeastern Spain (Bernal et al., 2022). Recent modeling for catchments in contiguous US
645 suggests that the NEP accounts for $\sim 7\%$ of the total evasion (Maavara et al., 2025). The net planktonic CO₂/exchange
646 fractions ($<5\%$) reported herein are even smaller than any fraction obtained in previous studies. Such a low range of net
647 planktonic CO₂ exchange fluxes is in great contrast to most of the examples obtained elsewhere, even for the site (BNE)
648 located nearest to the shoreline or with the greatest stream order (SO = 5). The pattern suggests that steep topography and
649 rapid erosion associated with active mountain building limit the development of soils and enable a scarce supply of
650 nutrients and rapid transit of river water, all of which apparently restrain the expression of planktonic metabolisms. In
651 contrast, voluminous amounts of metamorphic rocks are fractured and eroded, providing unlimited materials readily for
652 the oxidation of pyrite and petrogenic carbon mediated by microbial processes in the subsurface and riverbanks (Wang et
653 al., 2024; Lien et al., 2025). In consequence, CO₂ generated by these weathering reactions is drained into the river along
654 with groundwater flow and dominates over the contribution from other sources.

655 656 **4.6 Seasonal and spatial dynamics of microbial communities**

657 Variations in community diversity and composition were evident across different sites and sampling periods. Beta
658 diversity analysis indicated that microbial community compositions generally varied over time across most sites (Fig. 5).
659 Samples collected during the dry season were clustered, with the exception of BNE, and moderately deviated from those
660 collected during the wet season. Based on these ordination patterns and a detailed taxonomic analysis, the sites can be
661 categorized into three distinct groups: those exhibiting clear seasonal patterns (MLL01, CWL01, LY04), site with
662 complex temporal dynamics that do not strictly follow seasonal variation (DL), and the most downstream site (BNE)
663 where communities for different months are scattered at a great extent.

MLL01 and LY04 exhibited the most pronounced seasonal trends in community diversity and composition (Figs. 4 and 5). Community diversity was lower during the wet season than the dry season, except for the one in January at MLL01 (Fig. 4a). During the dry season, communities at both sites were predominantly composed of lithoautotrophs (Table S7). More specifically, MLL01 and LY04 were dominated by iron-oxidizing *Sideroxydans*, and sulfur-oxidizing *Thiothrix*, respectively. Both genera utilize the Calvin-Benson-Bassham cycle for carbon fixation (Emerson et al., 2010, 2013; Grabovich et al., 2023; Ravin et al., 2021). As dark DIC uptake rates were low at these sites during the dry season (Fig. 3b; Section 4.3), the abundant lithoautotrophic taxa suggest weak expression of their functions. Major members in communities appeared to shift towards different compositions in the wet season, such as autotrophic *Sulfuricurvum* and *Thiobacillus* (sulfur-oxidizing) at MLL01 and a combination of autotrophic and heterotrophic taxa at LY04 (*Thiobacillus*, *Arenimonas*, and Coxiellaceae). The wet-season shifts coincided with the highest dark DIC uptake rates measured in this study (Section 4.3), indicating that the wet-season lithoautotrophic communities were both abundant and functionally active. The presence of heterotrophic Coxiellaceae during the wet season coincided with increased TSM and POC levels, suggesting their introduction through terrestrial runoff rather than *in situ* growth and the impact of hydrological changes on community compositions.

CWL01 was characterized by high community diversity during the sampling period except in January (Fig. 4a), and showed a distinct seasonal pattern in a way that lithoautotrophic genera (*Sulfuricurvum* and *Thiobacillus*) became more abundant in August, while Cyanobacteria dominated throughout the dry season (Fig. 4b). This interpretation partially aligns with the low dark DIC uptake rates (Fig. 3). The patterns also suggest that abundances of lithoautotrophic and photosynthetic communities are complementary across different seasons, demonstrating significant metabolic versatility within the community.

DL was characterized by an intricate temporal pattern that did not follow the distinct dry-wet seasonal variations, as described previously. Community diversity remained relatively stable across the seasons (Fig. 4). The communities during the dry season (January, March, and May) clustered more tightly than the others (Fig. 5) and were predominantly composed of Cyanobacteria and *Thiothrix*, along with various heterotrophs (e.g., Comamonadaceae and Microscillaceae). The community in August also resembled those in the dry season and was dominated by *Thiothrix* and *Nitrospira*, highlighting the importance of sulfur and nitrogen metabolisms. However, the community in July deviated from this cluster and was dominated by the heterotrophic *Bacillus*. The temporal variability was correlated with DOC concentration, suggesting that organic matter availability and hydrological circulation rather than season per se drive community turnover at this site.

The most downstream site, BNE, was notably distinct from all other mountainous sites. It was characterized by relatively low community diversity throughout the sampling period (except for January) (Fig. 4) and high community variation between campaigns. (Fig. 5). The communities for March and May were dominated by *Fluviicola*, a genus specializing in degrading complex organic compounds (Woyke et al., 2011). For July and August, the community compositions shifted to the predominance of *Turicibacter* and *Polynucleobacter*, both of which are capable of decomposing dissolved organic matter (Bossard et al., 2002; Watanabe et al., 2012). The prevalence of heterotrophic taxa throughout all seasons was consistent with a high glycine catabolic rate (Fig. 3) and a significant correlation between community variation and DOC concentration (Fig. 5). The pattern also reflects its downstream role, where organic matter accumulated along the drainage path facilitates heterotrophic respiration.

Overall, chemolithoautotrophs, such as *Sideroxydans*, *Thiothrix*, *Sulfuricurvum*, and *Thiobacillus* were abundant during the wet season, whereas phototrophs and heterotrophs dominated during the dry season at the mountainous sites. For

704 BNE, the abundant heterotrophs (*Fluviicola*, *Turicibacter* and *Polynucleobacter*) were observed throughout the seasons
705 even though the constituting genera were different. We noted that abundance and activity were not always tightly coupled
706 across the catchment. For example, lithoautotrophs were numerically abundant at MLL01 and LY04 during the dry
707 season, yet dark DIC uptake rates were low at these sites in dry months. The pattern suggests that the most abundant taxa
708 were not always the most metabolically active. This decoupling underscores that 16S rRNA gene-based abundance is a
709 static metric and does not directly resolve activity. Further studies based on RNA are warranted to identify actively
710 metabolizing community members.

711 **Conclusions**

712 This study elucidates the dynamic nature of planktonic river metabolisms in the Beinan River system. By supplementing
713 ¹³C-labelled substrates, our incubations yielded that all categories of metabolic rates were consistently higher during the
714 wet season than the dry season, and that heterotrophic rates were higher than autotrophic rates. The deduced net planktonic
715 metabolic CO₂ rates of 7.89×10⁷ mol yr⁻¹ account for less than 5% of the exchange of CO₂ at the air-water interface,
716 pyrite-induced weathering, and petrogenic carbon oxidation. Microbial community compositions shifted from
717 chemolithoautotrophic sulfur- and ammonia/nitrite-oxidizing taxa in the wet season to a mixture of phototrophs and
718 heterotrophs in the dry season, reflecting the dynamic response of biological processes to variations in hydrology and
719 particle loading in the river. Although the study is inherited with several methodological limitations, future work should
720 pursue high-frequency event-driven monitoring, kinetic experiments to constrain *in situ* substrate uptake, RNA-based
721 approaches to identify actively metabolizing community members, and parallel benthic and hyporheic measurements.
722 Nevertheless, characterization of the water-column compartment remains essential for tracking upstream microbial and
723 geochemical signals and for predicting downstream fluxes to estuarine environments. Our findings highlight the
724 complexity and dynamics of planktonic river metabolisms and microbial communities in tectonically active mountainous
725 catchments prone to intense hydrological events and rapid landscape changes.

726 **Author contribution**

727 Jhen-Nien Chen: conceptualization; formal analysis; investigation; methodology; writing—original draft. Pei-En Chen:
728 conceptualization; methodology; formal analysis; writing—original draft; Yu-Shiang Yen: methodology; formal analysis;
729 investigation. Tzu-Hsuan Tu: conceptualization; methodology; writing - review & editing. Lu-Yu Wang:
730 conceptualization; methodology; data curation. Wan-Yin Lien: conceptualization; formal analysis; writing - review &
731 editing. Yueh-Ting Lin: conceptualization; data curation; writing - review & editing. Pei-Ling Wang: Conceptualization;
732 funding acquisition; investigation; methodology; project administration; resources; supervision; validation; writing—
733 review and editing.

734 **Competing interests**

735 The authors declare that they have no conflict of interest.

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