



The impact of heterotrophic bacteria on recalcitrant dissolved organic carbon formation in a typical karstic river

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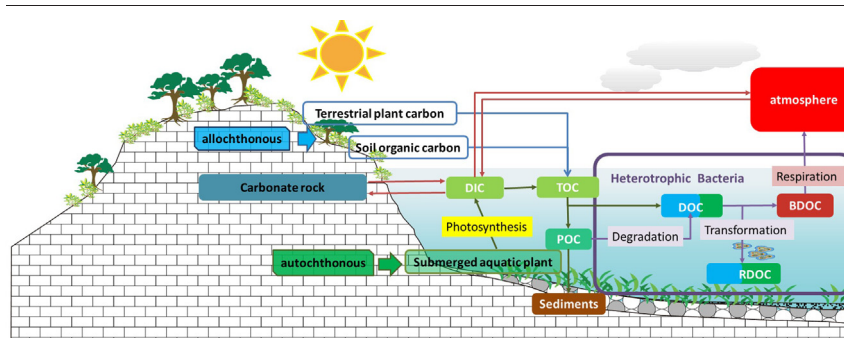
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HIGHLIGHTS

- Carbon isotopes and bacteria diversity were investigated to study karst carbon cycling.
- Autochthonous RDOC accounted for more than half of the RDOC increment in Lijiang River.
- Heterotrophic bacteria dominated the autochthonous microbial sourced DOC formation.

GRAPHICAL ABSTRACT



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ABSTRACT

Recalcitrant dissolved organic carbon (RDOC) resulting from microbial carbon (MCPs) holds promise as a relatively long-term natural carbon sink in marine environments. However, the RDOC formation mechanism remains uncertain in terrestrial aquatic systems. To determine the microbial impacts on autochthonous dissolved organic carbon (DOC), RDOC formation, and the critical influencing bacteria species, spatial changes in hydrochemistry, carbon isotopes, and microbial diversity were investigated in water samples from the karstic Lijiang River, southwest China. Samples were collected at various locations along the river system in May and July 2017. The biodegradable DOC (BDOC), RDOC, soil sourced DOC (SDOC), submerged aquatic vascular plant sourced DOC (PDOC) and microbial sourced DOC (MDOC) were calculated using the in-situ microbial incubation method, stable carbon isotopes and C/N ratio. RDOC accounted for 67% to 93% of DOC concentrations, measuring 1.3 mg/L and 1.2 mg/L in May and July, respectively. In May, BDOC concentrations increased by 0.05 mg/L from 0.18 mg/L to 0.23 mg/L, but decreased by 0.43 mg/L from 0.66 mg/L to 0.23 mg/L in July. The spatiotemporal variation of BDOC indicated photosynthesis was the main BDOC source and induced high autochthonous DOC formation, especially in May. However, RDOC was the dominant accumulation component in Lijiang River. MDOC increased by 0.86 mg/L from 0 to 0.86 mg/L in May and 0.78 mg/L from 0.10 mg/L to 0.88 mg/L in July, which was the dominant accumulated DOC and RDOC component. The abundance of *Sporichthyaceae* accounted for 3.4%–22.6% in May and *Novosphingobium* accounted for 3.5%–34.0% in July. These were the critical bacteria species induced MDOC formation, which were confirmed by their abundances in KEGG pathway modules determined by PICRUAST2. These results demonstrate that heterotrophic bacteria dominate autochthonous DOC and RDOC formation in the karst surface river, which is valuable for understanding organic carbon cycling in karstic aquatic systems.

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1. Introduction

The “missing” carbon sink in terrestrial ecosystems was calculated as 1.0–1.5 Pg C/a (Ciais et al., 2013; Melnikov and O'Neill, 2006; Regnier et al., 2013). In inland aquatic ecosystems, the net photosynthetic uptake of dissolved inorganic carbon (DIC) by aquatic organisms is 0.233 Pg C/a (Liu et al., 2010). The biological carbon pump (BCP), which affects the uptake of DIC in carbonate weathering and forms autochthonous organic carbon (AOC) via aquatic photosynthesis, is enhanced by high concentrations of DIC in karst areas (Chen et al., 2017; Einsele et al., 2001; Lerman and Mackenzie, 2005; Liu, 2013; Maavara et al., 2017; Noges et al., 2016; TERNON et al., 2000; Yang et al., 2016). Previous studies estimated that AOC in karstic aquatic systems accounted for 45–65% of the total organic carbon (Yang et al., 2016; Huang et al., 2020; Sun et al., 2021). However, these results were calculated based on the composition of homologous particulate organic carbon (POC) but not the composition of DOC directly, which would induce error on the estimation of DOC carbon sink.

DOC accounts for more than 80% of the TOC, especially in clear karst water, and is the dominant organic component in karstic aquatic ecosystems. As such, DOC composition, formation, and transformation is important for calculating autochthonous DOC and accurately determining organic carbon sink in karstic aquatic ecosystems (Noges et al., 2016). The origin and composition of DOC is closely associated with microbes (He et al., 2021), but problem remains on critical microbial species and their formational processes of microbial sourced DOC.

Microbial carbon pumps (MCPs) were identified in research of recalcitrant dissolved organic carbon (RDOC) in marine systems. MCPs have been considered as a critical mechanism for the uptake of dissolved organic carbon (DIC) and forms RDOC (Longhurst and Harrison, 1989; Jiao et al., 2014; Sanders et al., 2014; Legendre et al., 2015). According to the MCP effecting model, photoautotrophic bacteria are dominated by cyanobacteria in marine systems, and also the contribution of DIC up taken and microbial autochthonous DOC and RDOC formation (Johnson and Sieburth, 1979; Jiao et al., 2010). Aerobic anoxygenic phototrophic bacteria (AAPB), rhodopsin-containing proteobacteria, heterotrophic bacteria, and archaeal viruses reconstitute degradable DOC into microbial autochthonous DOC (Suttle, 2007; Zheng et al., 2011). In karstic aquatic systems, RDOC accounted for 50–70% of TOC (Li et al., 2018). In addition, autochthonous DOC forming bacteria species, such as AAPB, rhodopsin-containing bacteria, heterotrophic bacteria, cyanobacteria, and archaea, could also be found (Farquhar et al., 1989; Guy et al., 1993; Federherr et al., 2014; Barber et al., 2017). Thus, it can be inferred that bacteria play a key role in the formation of autochthonous RDOC in karstic aquatic systems. However, further research of RDOCs in karstic systems is required to identify critical bacteria species, track microbial RDOC forming process and calculate microbial RDOC fraction and flux.

Carbon stable isotope signatures function as unique tracers in aquatic systems and can be used to track sources and transformations between various carbon pools. An increase in $\delta^{13}\text{C}$ value occurs during carbon transformation and fixation, which induces the $\delta^{13}\text{C}$ variation resulting from different initial carbon sources and transforming processes (Shi et al., 2011; Koblížek, 2015; Viswanathan et al., 2016). Natural changes in $\delta^{13}\text{C}_{\text{DIC}}$ and $\delta^{13}\text{C}_{\text{DOC}}$, combined with C/N ratio in water can be exploited to determine the origins of the fractions from different carbon sources, such as organic carbon derived from terrestrial or aquatic, plants or aquatic microbes (Salka et al., 2014; Ferrera et al., 2017). The spatial dynamics of sources of DOC fractions along the karstic aquatic system can reveal the change and formation of DOC and RDOC.

The Lijiang River, a typical karst river located in Southwest China, was chosen to investigate the autochthonous DOC formation process. The river is characterized by a high DIC concentration and flourishing submerged plants and algae. Water samples were collected in rainy July when concentrations of allochthonous organic matter were higher and in May when the temperature and solar radiation was similar to July, however, allochthonous organic matter was lower. The hydrochemistry, carbon isotopes, and bacterial community diversity were measured at six locations along the

river, to identify spatial variations in the origin and composition of DOC and dominant bacteria species. In this study, we aim to determine: (1) the evidence that microbes participate in autochthonous DIC-DOC formation and DOC-RDOC reconstitution, and (2) the important bacteria species that involved in autochthonous organic matter reconstitution.

2. Materials and methods

2.1. Study area

The Lijiang River is located in northeastern Guangxi Province, China. It is 164 km long with the watershed of 12,690 km² (Fig. 1). The river is in the upper region of the Gui River, belonging to the Pearl River System and flows south from the Yuecheng Mountains. It has a subtropical humid monsoon climate with an annual average temperature range of 16.5–20.0 °C and an annual average precipitation of 2000 mm. The vegetation in the Lijiang watershed is dominated by evergreen broad-leaved forests and C₃ plants. The watershed is underlain by Paleozoic Devonian and Silurian strata, of which the upper region, which extends from the river source to Guilin city, flows over granite and clastic rocks. The geology of the mid-downriver catchment area is dominated by carbonate rocks with a thickness of about 900–3000 m, accounting for approximately 65% of the total catchment. Given the combination of the pure thick carbonate rock layer, the geo-background, and the hot and humid monsoon climate, the environment is classified as a typical hoodoo-plain karst landscape.

The Lijiang River is characterized by good water quality, with warm (about 19 °C) water and a horizontal Secchi disk depth of tens of meters. These features, combined with abundant light, permit the growth of dense submerged aquatic vegetation on the riverbed. Vegetation varies with respect to the geomorphological features of the river. Typical native submerged C₃-macrophytes originating from the surface waters of the South China Sea can be found in the main channel. These include, *Vallisneria natans* L., *Potamogeton distinctus* A. Benn., *Ceratophyllum demersum* L. and *Hydrilla verticillata*, which represent 90% of the submerged aquatic vegetation. Other primary producers include a variety of filamentous benthic algae, as well as emergent and floating species that can become dominant in the channel margins.

2.2. Sampling

Six water samples were collected from the mainstream of the Lijiang River in both May and July 2017. Sample sites L1 and L2 are in non-karst and covered karst areas, respectively, while sites L3–L6 are located in exposed karst areas (Fig. 1).

Discharge data for the Lijiang River and its tributaries was obtained from hydrological stations operated by the Guilin Hydrographic Office (He et al., 2021). Water temperature, pH, Electronic Conductivity (EC, at 25 °C), and Dissolved Oxygen (DO) (accuracy: 0.01 °C, 0.01 pH, 1 $\mu\text{S cm}^{-1}$ and 0.1 mg/L, respectively) were measured using field water kit (POSEL, France). Bicarbonate (HCO_3^-) was measured in the field using an alkaline testing kit (0.1 mmol/L, Merck, Germany).

In addition, water samples (100–2000 mL) were collected to determine major ion, total organic carbon (TOC) and DOC concentrations, and $\delta^{13}\text{C}_{\text{DIC}}$. Samples were collected after rinsing 3–5 times and then stored at 4 °C before analyses. The water samples were triple-filtered using 0.22 μm micro-membrane and separated into 250 mL volumes using brown glass bottles (burned at 300 °C for 3–4 h to remove all organic matter) for RDOC analysis. Particulate organic carbon (POC) samples were collected by glass fiber membranes with a 0.77 μm mesh size (burned at 300 °C). Meanwhile, 400 mL of water was filtered using a sterilized micro-membrane (0.22 μm mesh size, Millipore, USA) to collect the microbes present for DNA extraction. Microbe-containing micro-membranes were kept in the sterilized tubes and were stored in ice bags at -20 °C until DNA extraction.

In total, two carbonate rock samples, four soil samples, three samples of terrestrial plants, four samples of submerged aquatic plants (*Vallisneria*

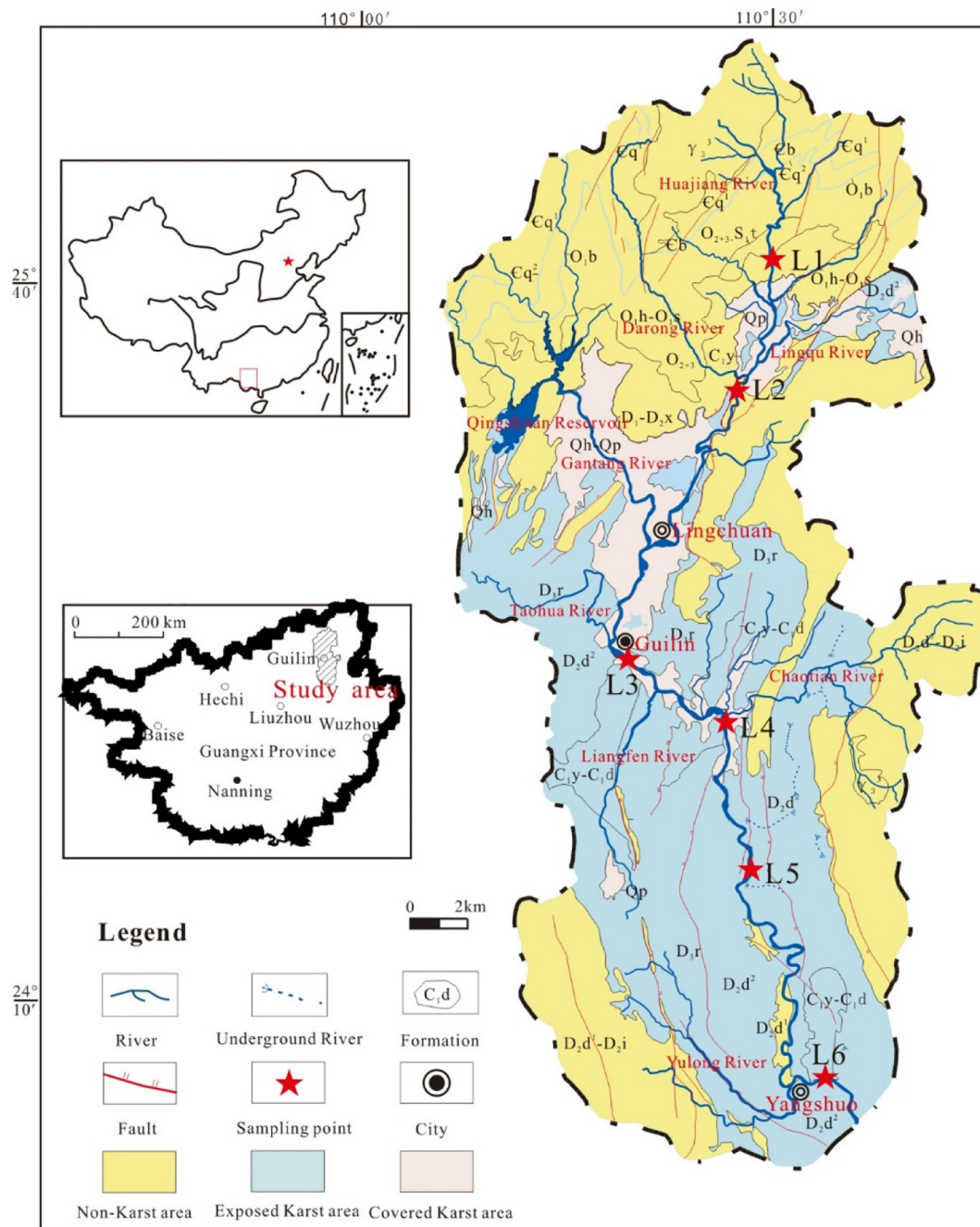


Fig. 1. Geological map of Lijiang River catchment, with sampling sites identified by red stars. Inset images show the location of the river system in relation to China.

natans L., *Potamogeton distinctus* A. Benn., *Ceratophyllum demersum* L., and *Hydrilla verticillata*), and three algae samples were collected at each sampling site where possible. Microalgae were also collected by filtering 700 mL of water. All samples were dried in a vacuum refrigerating machine and were crushed to with a diameter of 0.15 mm for further stable isotope analysis.

2.3. Hydrochemical analysis

Concentrations of SO_4^{2-} , NO_3^- , and Cl^- in the water samples were analyzed using an ICS-900 anion chromatography system (Thermo Fisher Scientific, USA, accuracy of 0.01 mg/L). Concentrations of Ca^{2+} , Mg^{2+} , K^+ , and Na^+ were analyzed in the ICP-OES Optima 2100DV system (Perkin Elmer, USA, accuracy of 0.01 mg/L). Concentrations of TOC, DOC, and

RDOC were analyzed using a Multi C/N 3100 analyzer (Analytik Jena AG, Germany, accuracy of 0.001 mg/L).

RDOC concentration was determined following the method of Young et al. (2005). One percent of in-situ autochthonal microbes was extracted using a 0.22 μm micro-membrane, resuspended in the same volume of de-ionized water, before addition to each brown bottle. The brown bottles were incubated at 28 °C for 30 days. As reported, the CDOM Excitation and Emission Matrix (EEM) fluorescence spectrum revealed that the labile organic carbon component, a small protein-like component, disappeared after incubation (Liu, 2018). In the 180-day incubation experiment, concentrations of DOC plateaued at a stable low level after 30 days incubation (Li et al., 2018). Thus, after 30 days incubation, the heterotrophic bacteria have consumed liable DOC in karst aquatic water samples and produced RDOC. All incubations were re-filtered using 0.22 μm micro-membrane

before analysis in the Multi C/N 3100 analyzer. Hydrochemical parameters and organic carbon concentrations were detected at Chongqing Key Laboratory of Karst Environment & School of Geographical Sciences, Southwest University, Chongqing, China.

50 mL sub-samples were collected and preserved in parafilm-sealed plastic containers to analyze the $\delta^{13}\text{C}_{\text{DIC}}$. Before sealing, two drops of mercury chloride (HgCl_2) were added to prevent microbial degradation. These samples were analyzed for DIC, DOC, $\delta^{13}\text{C}_{\text{DIC}}$, and $\delta^{13}\text{C}_{\text{DOC}}$ at the Third Institute of Oceanography, State Oceanic Administration, Xiamen, China using an OI Analytical "TIC-TOC" Analyzer and a Finnigan Mat Delta Plus isotope ratio mass spectrometer, following the procedure outlined by St-Jean (2003). The $\delta^{13}\text{C}$ of bedrock, soil organic matter, and terrestrial/aquatic plant samples were analyzed at Chinese Academy of Agricultural Sciences (CAAS), Beijing, China, using the Isoprime isotope ratio mass spectrometer (Isoprime 100, Germany). These samples were analyzed alongside bracketing standards and the standard deviations of isotopic samples was measured at $\pm 0.2\%$. All carbon isotope values were reported against the Vienna Pee-Dee Belemnite (the international reference standard for carbon isotopes), as VPDB‰. Total carbon and total nitrogen in the water, soil organic matter, and terrestrial/aquatic plants samples were simultaneously measured (accuracy of 0.01 mg/L), with the results used to calculate C/N ratios.

2.4. Microbial community analysis

DNA of the microbial community was extracted using a Fast DNA Spin Kit for Soil (MP Biomedicals, Biomedicals, Santa Ana, CA, USA) and subsequently, sequenced using an Illumina MiSeq platform (MajorBio Co. Shanghai, China). Sequencing was performed according to the manufacturers' standard protocols using the original V3-V4 primers, 338F (5'-ACTCCTACGGGAGGAGCA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'), to sequence the 16S rDNA and analyze microbial community diversity. A total of 272,709 sequences from 12 samples were identified and had an average length of 440 bp. QIIME, a microbiome bioinformatics platform, was used to reduce the number of erroneous sequences and resulted in 808 unique sequences, which were considered to represent the operational taxonomic units (OTUs). Reads occurring fewer than twenty times in the entire data set were removed. Chimeras and single reads were removed by the USEARCH pipeline. Each read was classified as an OTU by 97% similarity, and representative sequences were randomly chosen from each OTU. OTUs matched the Greengenes database with an identity percentage of greater than 97%.

2.5. Statistical analyses

A subsample OTU table was created according to the minimum sequence number. Statistical analyses were then performed in the i-Sanger iCloud server (<http://www.i-sanger.com/>) developed by the MajorBio Company (Shanghai, China) based in R and QIIME. Community heatmap and cluster analyses were used to determine the similarity of bacterial community diversity in different samples. Spearman's rank correlation heatmap and cluster analyses were also performed on genus level taxonomic ranks to determine the correlations between the top fifty genera and environmental parameters. All taxonomic ranks were included in this analysis to determine the best resolution level for each rank. Plots were generated from the i-Sanger iCloud server and were edited using AI CS4 and Corel Draw X4 applications.

3. Results

3.1. Hydrochemistry and $\delta^{13}\text{C}_{\text{DIC}}$ characteristics

Ca^{2+} and HCO_3^- were the most abundant cation and anion, respectively, and the hydrochemical water type was HCO_3^- -Ca. The pH values increased from 7.10 to 8.13 (mean = 7.49) in May and from 6.99 to 7.27 (mean = 7.16) in July. The concentrations of Ca^{2+} in the water samples

increased from 8.8 mg/L to 47.7 mg/L (mean = 29.8 mg/L) in May and from 4.6 to 38.1 mg/L (mean = 21.0 mg/L) in July (Supplementary Fig. S1). Similarly, the HCO_3^- concentrations increased from 0.3 to 2.2 mmol/L (mean = 1.4 mmol/L) in May and from 0.2 to 1.8 mmol/L (mean = 1.0 mmol/L). The $\delta^{13}\text{C}_{\text{DIC}}$ values for water samples ranged from -12.9% to -10.0% (mean = -10.9%) in May and from -12.5% to -9.7% (mean = -11.0%) in July (Fig. 2). In May, concentrations and mean values of the hydrochemical parameters were higher than those in July. Especially, the concentrations of Ca^{2+} and HCO_3^- which were 38.6 mg/L and 1.9 mmol/L in May, but 23.6 mg/L and 0.9 mmol/L in July.

3.2. Variations in organic carbon in the Lijiang River

TOC is primarily composed of DOC in Lijiang River, and the concentrations of DOC and TOC were higher in July than in May. The mean value of TOC concentration was 1.8 mg/L in May (0.7–2.3 mg/L), which was 51% of that in July (3.0–4.3 mg/L, mean 3.5 mg/L) (Fig. 2). The mean value of DOC concentration was 1.6 mg/L in May (0.6–2.1 mg/L), which was 70% of that in July (1.6–3.0 mg/L, mean 2.3 mg/L). RDOC accounted for 67–93% of the DOC concentration. Comparatively, BDOC accounted for 7–33% of the DOC concentration (Fig. 2).

Organic carbon concentrations revealed a substantial increasing trend from upriver to downriver, with a higher concentration observed in July rather than May. The TOC and DOC concentrations increased by 186% and 234% in May respectively, but only 10% and 25% in July. The RDOC concentration increased by 427% in May but only 47% in July. In contrast, the concentration of BDOC revealed fluctuant dynamics, which showed 27% increase in May but 37% decrease in July (Fig. 2).

3.3. The contributions of different OC sources to DOC in the Lijiang River

The $\delta^{13}\text{C}_{\text{DOC}}$ values and C/N ratios also showed an increasing trend along the river, with concentrations higher in May than in July (Fig. 2). In May, the $\delta^{13}\text{C}_{\text{DOC}}$ values increased from -26.7% to -22.6% (mean = -24.3%), and the C/N ratios increased from 9.8 to 12.8 (mean = 11.6). In July, the $\delta^{13}\text{C}_{\text{DOC}}$ values increased from -25.4% to -23.1% (mean = -24.3%), and the C/N ratios increased from 10.4 to 12.1 (mean = 11.2) (Fig. 2). Theoretically, two DOC sources, namely, allochthonous OC (soil organic carbon) and autochthonous OC (submerged aquatic plants and microalgae/bacteria), could be the main contributors of DOC. Due to different OC sources having distinctive $\delta^{13}\text{C}_{\text{DOC}}$ values and C/N ratios (Thornton and Mcmanus, 1994; Meyers, 1997), the contributions of submerged aquatic plants, microalgae/bacteria, and soil organic matter in Lijiang River waters have been estimated. This data can be found in Supplementary Tables S1 and S2.

As shown in Fig. 3, allochthonous OC was the major upriver source of DOC, accounting for 63% in May and 72% in July. In contrast, further downriver, allochthonous OC remained the major DOC source in July (58%), but became a minority source in May, accounting for only 37% DOC (Fig. 3). Autochthonous OC became dominant in downriver samples in May (site L6), whereby microalgae/microbe sourced DOC (MDOC) accounted for 44% and submerged aquatic plant sourced DOC (PDOC) accounted for 19% (Fig. 3). The main source of DOC in Lijiang River differed between May and July.

The concentration of three kinds of OC component were calculated according to their fractions of total DOC concentration (Table S2). The highest concentrations of SDOC and PDOC were found in the mid-river in both May and July. SDOC concentration reveals fluctuating spatial variations through July. In comparison to site L1, the PDOC concentration increased from 0.21 mg/L by 0.17 mg/L to 0.38 mg/L at site L6 in May, but decreased from 0.55 mg/L by 0.15 mg/L to 0.40 mg/L at site L6 in July. The MDOC concentration increased from 0 mg/L to 0.86 mg/L in May and 0.10 mg/L to 0.88 mg/L in July (Table S2).

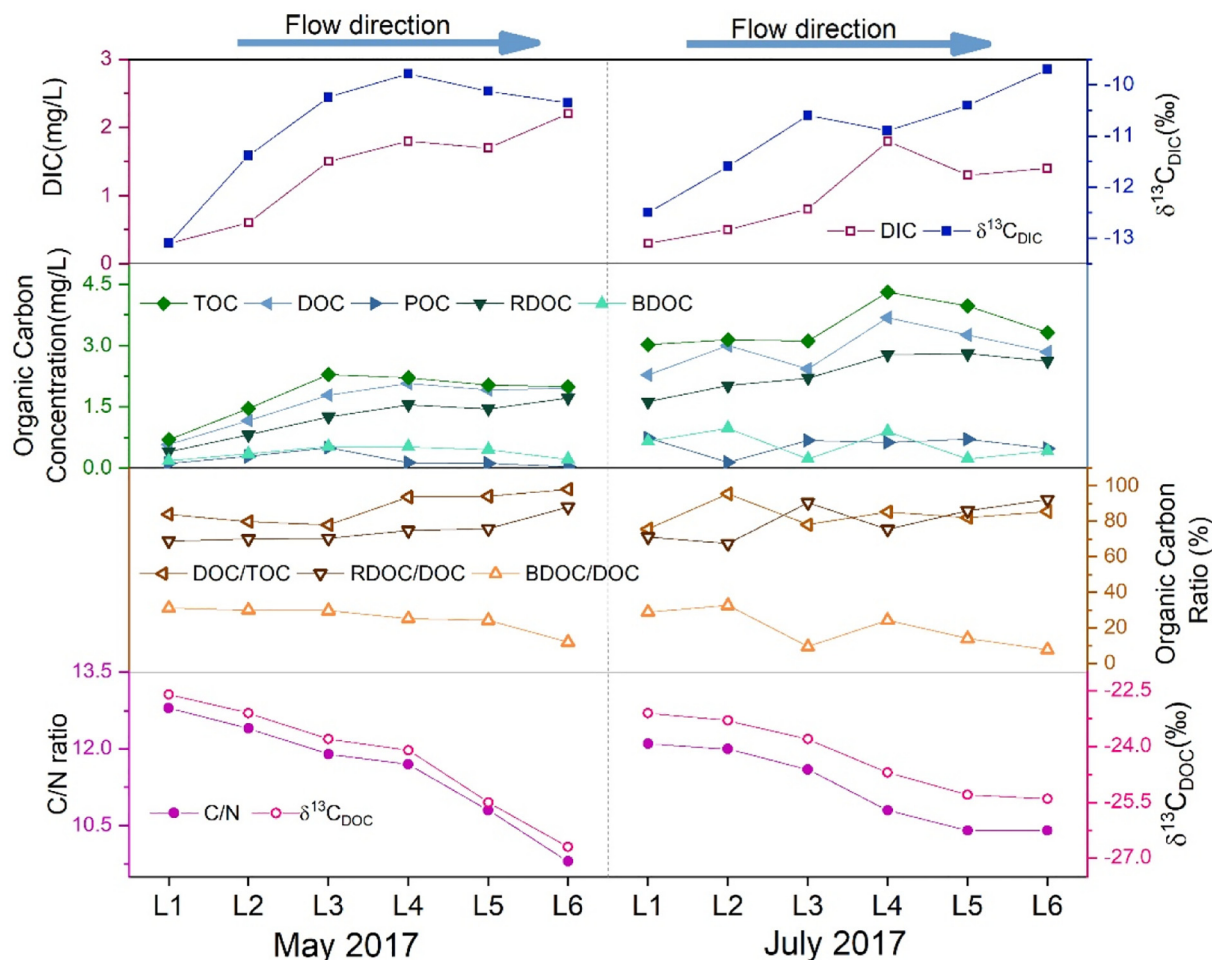


Fig. 2. Dynamics of concentration and $\delta^{13}\text{C}$ isotopes of inorganic and organic carbon components in Lijiang River.

3.4. Variations of bacteria diversity in the Lijiang River

Three main phyla, which included *Actinobacteria*, *Proteobacteria* (such as *Alpha*-, *Gamma*- and *Delta*-*proteobacteria*), and *Bacteroidetes* accounted for more than 95% of the total OTU numbers in Lijiang River (Fig. S2). Twenty-five genera have high relative abundances and belong to 13 families, which accounted for 50–82% of total OTU numbers (Fig. 4).

Figs. 4 and 5 show obvious differences in microbial community diversity at the genus and OTU level from the upper to mid-downriver in the studied months May and July. In May, the most abundant genera were *Limnohabitans* and unclassified_f_Burkholderiaceae which belong to the family Burkholderiaceae (*Gammaproteobacteria*); and unclassified_f_Sporichthyaceae, norank_f_Sporichthyaceae, and hgcI_clade that belong to the family Sporichthyaceae (*Actinobacteria*). Relative abundances of *Limnohabitans* and unclassified_f_Burkholderiaceae continuously decreased, from 26.7% to 7.8% and 16.7% to 3.5%, respectively. In contrast, the relative abundances of the family Sporichthyaceae increased from 3.4% to 22.6% (Fig. 4). In July, the relative abundances of Burkholderiaceae and Sporichthyaceae fluctuated, with less than 10% of each found in each sample (Fig. 4).

Inversely, in July, the most abundant genera were *Novosphingobium* and *Sphingobium* belonging to family Sphingomonadaceae (*Alphaproteobacteria*), and *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* belonging to family Rhizobiaceae (*Alphaproteobacteria*). The relative abundances of most abundant family, *Sphingomonadaceae* and *Rhizobiaceae*, fluctuated along the Lijiang River. These fluctuations ranged from 7.6% to 49%. *Novosphingobium* accounted for 34.2% in the upriver samples (L1), and *Sphingobium* accounted for 15.0% in downriver samples (L6), and were

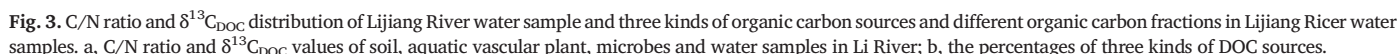
the dominant genera at these sample sites (Fig. 4). The relative abundances of genus *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* ranged from 2.3% to 11.0%. *Sphingomonadaceae* and *Rhizobiaceae* were rarely found in samples collected in May (Fig. 4).

4. Discussion

4.1. Biological impacts on DIC

Vegetation in the Lijiang Watershed is mostly dominated by C_3 plants. $\delta^{13}\text{C}$ values of C_3 vegetation range from -28.6‰ to -24.7‰ (mean = -27.1‰ ; Table S3). Carbonate rocks have a mean $\delta^{13}\text{C}$ value of -0.6‰ in the Lijiang watershed (Table S3). In the Lijiang River, bicarbonate (HCO_3^-) is the dominant DIC species, as pH of water was within the range of 6–9. Therefore, as a dissolving product of the silicate or carbonate by CO_2 from C_3 vegetation under open system conditions, a $\delta^{13}\text{C}_{\text{DIC}}$ value around -13.7‰ was expected ($-27.1\text{‰} + 4.4\text{‰}$ [fractionation between CO_2 and its soil organic matter (Cerling et al., 1991)] + 9‰ [fractionation between soil gas CO_2 and HCO_3^- (Deines, 2004)]).

The $\delta^{13}\text{C}_{\text{DIC}}$ values of water at L1 (upper river) were close to the theoretically calculated value, -12.9‰ and -12.5‰ , suggesting that DIC originated from silicate dissolution of C_3 vegetation by CO_2 . The elevated $\delta^{13}\text{C}_{\text{DIC}}$ in mid-downriver water samples (L3) indicated that most DIC resulted from carbonate dissolution of C_3 vegetation by CO_2 under open system conditions. However, $\delta^{13}\text{C}_{\text{DIC}}$ was influenced by other geochemical processes. Elevated $\delta^{13}\text{C}_{\text{DIC}}$ could be associated with carbonate dissolution by sulfuric and nitric acids, degassing, and biological processes (photosynthesis of subaquatic community). However, the relatively low



DOC can easily be broken down into refractory DOC (RDOC) and biodegraded DOC (BDOC) (Young et al., 2005). Similar RDOC concentrations were found in May and July (1.3 mg/L and 1.2 mg/L, respectively). The higher DOC concentrations in May can be attributed to higher concentrations of BDOC in Lijiang River. BDOC can be consumed by heterotrophic bacteria which dominate the bacterial population in surface river waters (Newton et al., 2011; Shabarova et al., 2014). This means that BDOC concentrations should decrease along further downriver in typical surface river waters, unless further BDOC addition occurred. The BDOC increase at mid-river in both May and July can be attributed to increased aquatic photosynthesis rather than from a microbial source, because the abundance of autotrophic bacteria were limited (Fig. 4). In addition, the concentration of BDOC increased by 0.05 mg/L in May but decreased by 0.4 mg/L in July across all study sites. These trends coincided with the DIC-DOC linear relationship where there was a higher autochthonous DOC formation rate in May than in July. Further downriver, the decreasing concentrations of BDOC indicated consumption of BDOC by heterotrophic bacteria and reconstituted into microbial sourced DOC. On the other hand, RDOC was the dominant DOC component in the Lijiang River due to its high concentrations. According to previous reports, RDOC in aquatic systems mainly originates from microbial DOC formation (Ogawa et al., 2001; McCallister and Bauer, 2004; Kawasaki and Benner, 2006; Jiao et al., 2010). Thus, increased concentrations of RDOC in the mid-downriver may induce microbial sourced DOC. However, this hypothesis requires further investigation by microbial analysis.

Fig 6 shows that DIC is significantly correlated with DOC both in May and July ($R^2 = 0.87$ and 0.64 , respectively), and the linear regression revealed the similar trend (0.74 and 0.75) but different intercepts (2.74 and

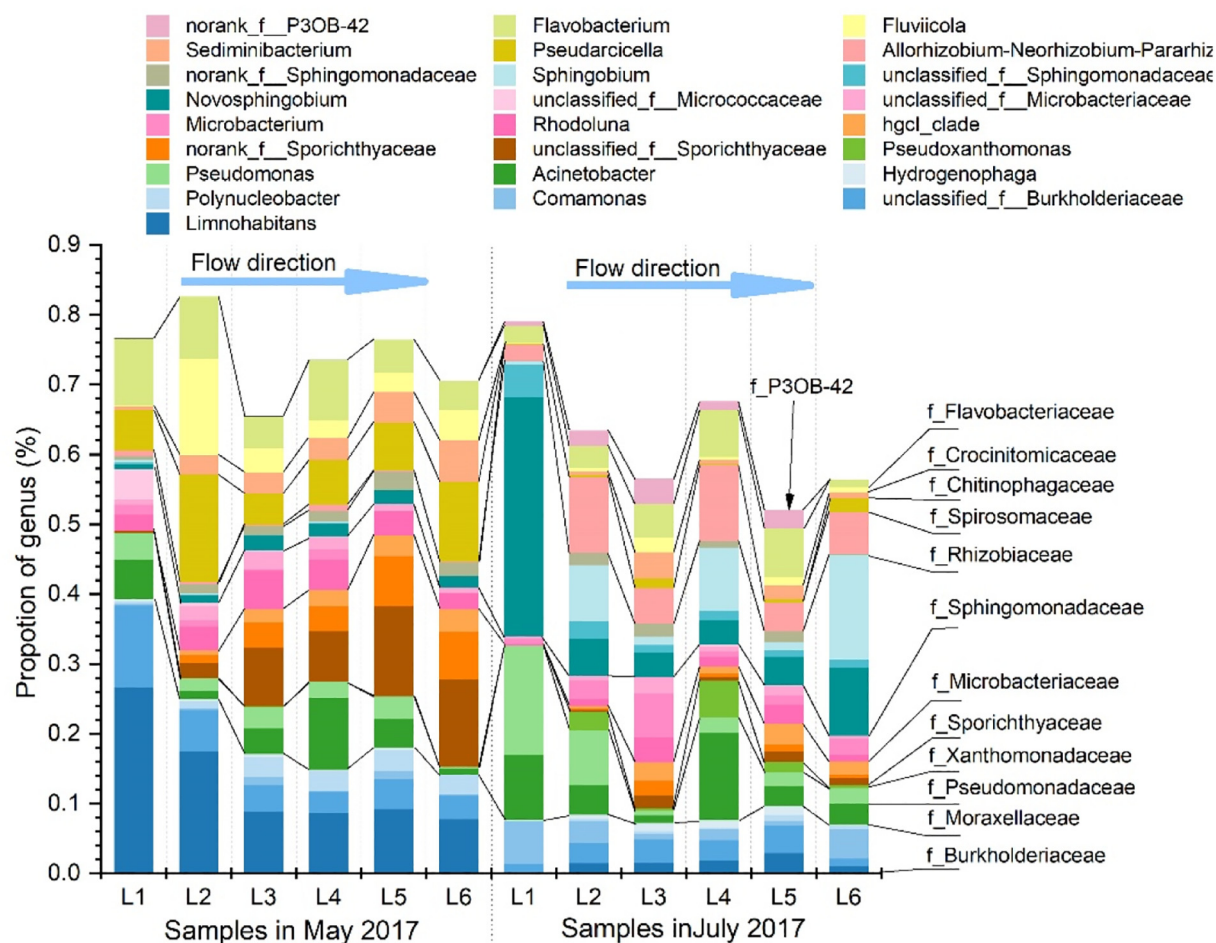


Fig. 4. Relative abundances of bacterial communities at the genus and family level in May and July for the Lijiang River. L1–L6 are the sampling sites.

4.3. Autochthonous DOC production in karst aquatic ecosystems

Three components of DOC in Lijiang River were calculated. SDOC dominated in the upriver part, whereas MDOC increased and became the

dominant organic carbon component in the downriver region, especially in May. As reported, allochthonous DOC was influenced by soil erosion, which fluctuated with precipitation and tributary recharge variations (Wang, 2013). Thus, SDOC concentration and fraction were higher in

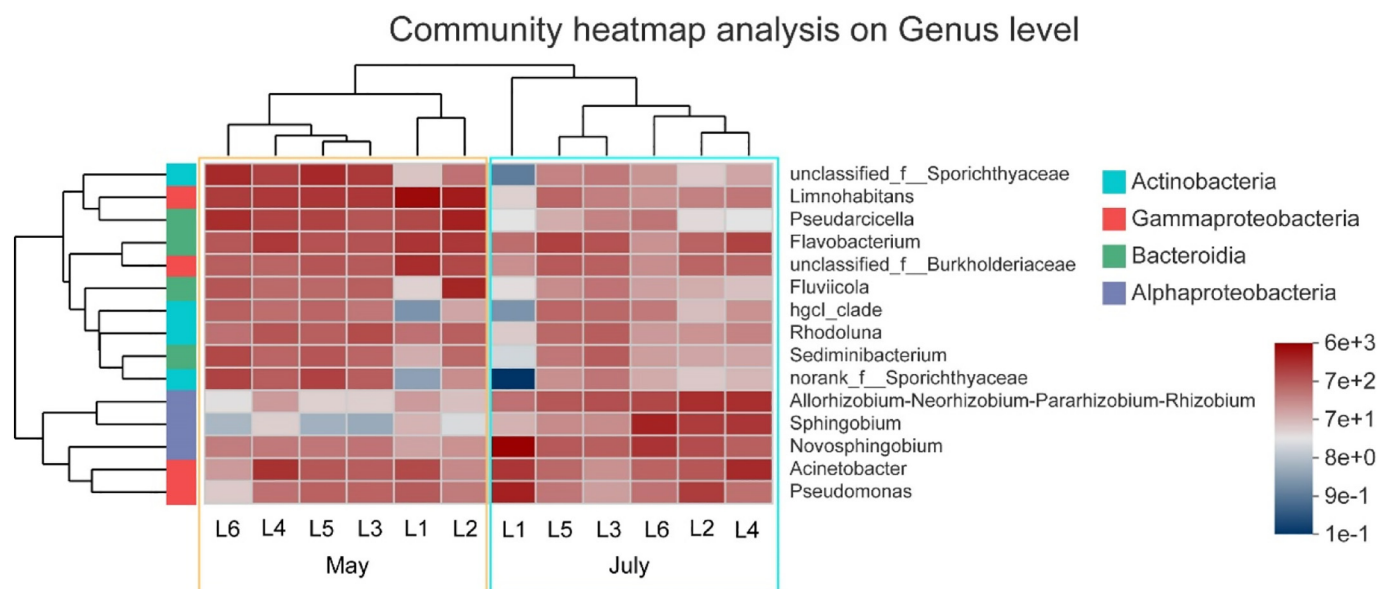


Fig. 5. Heatmap cluster plot of bacteria communities at the genus level in May and July for the Lijiang River. L1–L6 are the sampling sites.

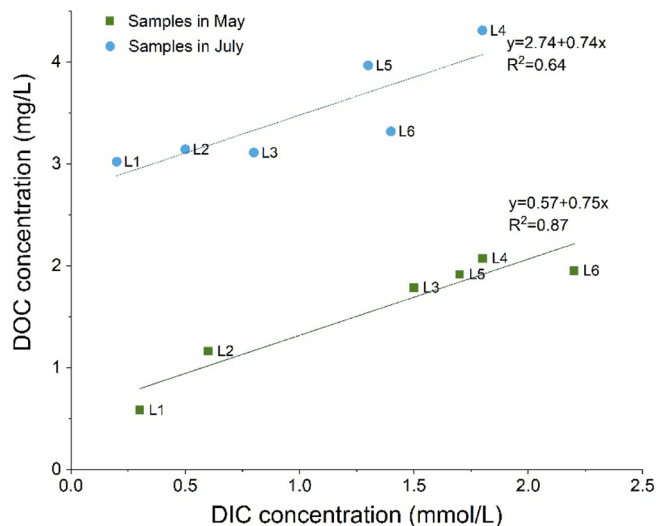


Fig. 6. Linear fit of DIC and DOC concentrations in Lijiang River in and July.

July than in May due to higher precipitation and turbidity recharge during summer (He et al., 2021). The proportions and concentrations of SDOC decreased from upriver to downriver, which indicated that the increase in DOC was caused by autochthonous DOC rather than allochthonous SDOC, especially in May.

The concentrations of PDOC showed an increase in the mid-river region where submerged aquatic plants flourished, whereas PDOC decreased in the downriver region where submerged aquatic plants became infrequent. The quantity and growth environment of aquatic plants were the main factors that influenced PDOC production (He et al., 2021), thus PDOC reveals the highest concentrations in the mid-river, with higher concentrations in May. In addition, PDOC was utilized by heterotrophic bacteria, which resulted in a decrease in PDOC further downriver in both May and July. Heterotrophic bacteria growth caused an MDOC increase. Accordingly, MDOC concentrations showed a continuously increasing trend along the river in both May and July, which contributed about 0.8 mg/L and 0.7 mg/L increase of autochthonous DOC, respectively. The increase of autochthonous DOC was attributed to MDOC accumulation in Lijiang River.

4.4. Microbial community variations associated with organic matter conversion

As seen in Fig. 4, the most dominant genera in Lijiang River were heterotrophs. Autotrophic bacteria, including *Cyanobacteria* and *Chloroflexi*, accounted for less than 1%, which indicates that MDOC was not produced directly from DIC. We suggest that heterotrophic bacteria used autochthonous DOC produced by aquatic plants and reconstituted it to form MDOC. Variations in bacterial diversity across May and July combined with the considerable relationships between the dominant taxa lineage and important environmental parameters could confirm this hypothesis.

The dominant genera in July, such as *Novosphingobium*, *Sphingobium* and *Sphingomonas* from family *Sphingomonadaceae* and *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* from family *Rhizobiaceae*, are heterotrophs and revealed a significant positive correlation with RDOC ($p < 0.05$) and SDOC ($p < 0.01$) (Fig. 7). Genera *Novosphingobium* and *Sphingobium* are often found in different soil samples (Newton et al., 2011; Glaeser and Kämpfer, 2014) and *Rhizobiaceae* is root nodule bacteria, which indicates a soil origin (Noel, 2009). In July, the discharge of the Lijiang River was approximately three times higher than in May for all sampling sites, which caused exaggerated soil erosion. As a result, high concentrations of soil materials, including organic matter and soil habituated bacteria, were flushed into the Lijiang River, which resulted in a higher SDOC concentration and a higher abundance of soil related bacteria in July. The soil sourced bacteria contributed to RDOC production.

May marks the beginning of the wet season, thus the discharge of Lijiang River is less influenced by strong precipitation, which is 35% lower than in July. The bacterial community is dominated by typical freshwater bacteria, such as family *Sporichthyaceae* and genus *Limnohabitans* (Newton et al., 2011; Jezberová et al., 2017; Linz et al., 2017). The relative abundance of *Limnohabitans* revealed a significant positive correlation with PDOC ($p < 0.05$) but not MDOC (Fig. 7). The relative abundance of *Sporichthyaceae* showed significant positive correlations with DIC and MDOC fractions. *Sporichthyaceae* and *Limnohabitans* are aerobic heterotrophic bacteria (Warnecke et al., 2005; Hahn et al., 2010a; Newton et al., 2011) that consume low molecular weight organic carbon (Newton et al., 2011; Kasalický et al., 2013; Vigneron et al., 2019). However, there are differences between *Sporichthyaceae* and *Limnohabitans*. *Limnohabitans* is typical heterotrophic bacteria, which consumes organic carbon for energy, and flourishes in BDOC-rich shallow lakes and pools (Lindström et al., 2005; Hahn et al., 2010b; Jezberová et al., 2017; Linz et al., 2017), where *Limnohabitans* dominates over other bacteria species. Thus, the upriver region is the optimal habitat of *Limnohabitans*, where the BDOC fraction was much higher than further downriver. High proportions of autochthonous PDOC are degraded by heterotrophic bacteria, which encourages the rapid growth of *Limnohabitans*. *Sporichthyaceae*, an *Actinobacteria*, has phototrophic properties and outcompetes in oligotrophic environments (Sharma et al., 2008; Jezberová et al., 2013; Ghylin et al., 2014; Nakamura et al., 2016). Thus, *Sporichthyaceae* dominates in the downriver region where BDOC concentrations and fractions were much lower. Photoheterotrophic bacteria use BDOC as a carbon source combined with solar energy to produce their own DOC, which indicates high reconstitution rates of BDOC produced by aquatic plants to MDOC. Heterotrophic microbes are the most important producers of RDOC in experimental simulation (Kawasaki and Benner, 2006) and marine systems (Dang, 2020). *Sporichthyaceae* is supposed to be an important source of MDOC and autochthonous RDOC in May; however, more evidence is required.

4.5. Microbial metagenomic functions demonstrate MDOC formation

There were 264 KEGG pathway modules determined by PICRUAST2, of which 57 modules connected with carbon metabolism function. There were 43 organic carbon synthesis modules, of which these abundances revealed significant correlations ($p < 0.05$) with the abundances of six dominant bacteria genera in Lijiang River (Fig. 8). The abundance of *Limnohabitans* revealed significant negative correlations with most organic carbon synthesis modules. The abundance of *Sporichthyaceae* genera revealed significant positive correlations with dTDP-L-rhamnose biosynthesis, trehalose biosynthesis, C10-C20 isoprenoid biosynthesis, coenzyme M biosynthesis, nucleotide sugary biosynthesis, formaldehyde assimilation, triacylglycerol biosynthesis, Calvin cycle and pyruvate oxidation. The abundance of *Novosphingobium* revealed significant positive correlations ($p < 0.05$) with reductive acetyl-CoA pathway, C4-dicarboxylic acid cycle, beta-oxidation, and malonate semialdehyde pathway, but negative correlations with organic carbon synthesis modules. Very few organic carbon synthesis modules had significant correlations with the abundances of *Sphingobium* and *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*. These results indicate that *Sporichthyaceae* played a key role in MDOC formation in the Lijiang River, particularly in May. *Novosphingobium* was revealed to cooperate with the carbon fixing function.

In the marine system, autotrophic bacteria are primary producers by phototrophic synthesis. Heterotrophic microbes transform labile DOC to RDOC, which is resistant to further biological degradation and preserved in the ocean for decades to millennia (Dang, 2020). Heterotrophic bacteria in karst aquatic systems perform a similar role of consuming BDOC and transforming it into RDOC. Heterotrophic microbes are the dominant proportion of the total microbial quantity, whereas autotrophic microbes are limited and play a minor role in organic carbon cycling of surface karstic aquatic systems. Submerged vascular aquatic plants are the primary organic producers in surface karst aquatic systems. In addition, the dominant heterotrophic bacteria species are seasonally variable and influenced by

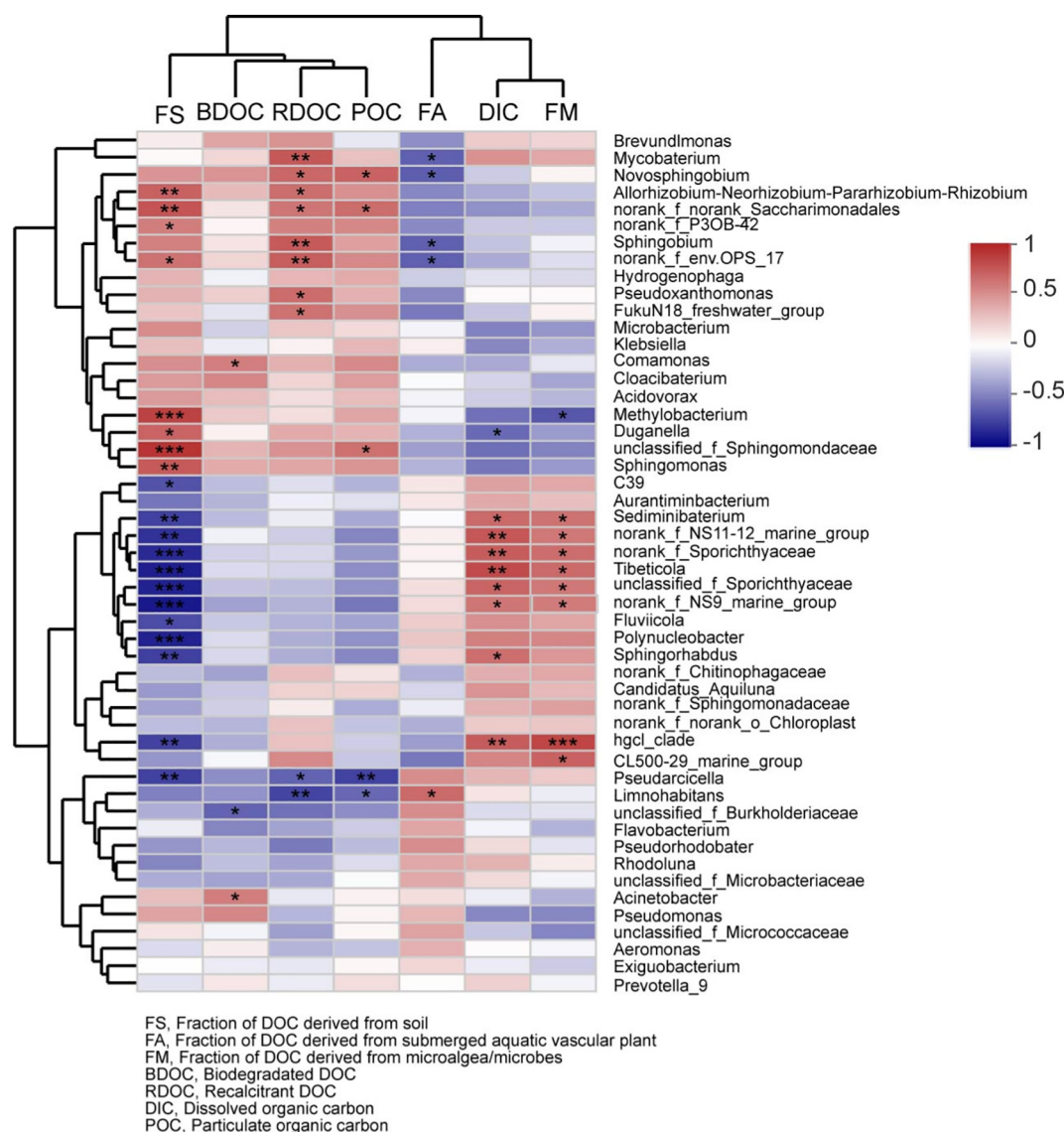


Fig. 7. Spearman correlation heatmap cluster of organic carbon component to the top 50 genera in the Lijiang River. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

allochthonous bacteria participation. The alteration of dominant heterotrophic bacteria species influences the MDOC formation mechanism and transformation rate. Future work should focus on specific MDOC formation mechanisms of different heterotrophic bacterial species and their MDOC and RDOC transformation rates.

5. Conclusion

In this study, water samples were collected in May and July to investigate the organic carbon composition and photosynthesizing-microbes that contribute to autochthonous organic carbon formation processes in a typical karstic surface river. Spatiotemporal variations in BDOC and RDOC concentrations identified aquatic plant photosynthesis as the main source of BDOC and PDOC, with heterotrophic bacteria contributing to BDOC consumption and RDOC formation. The fractions of three DOC sources were determined by $\delta^{13}\text{C}_{\text{DOC}}$ values and C/N ratios (Fig. 3). The increased fractions and concentrations of MDOC demonstrated that microbes contributed to PDOC consumption and became the main source of DOC and RDOC. Bacterial community diversity revealed that *Sporichthyaceae* and *Novosphingobium* were the dominant bacteria species influencing MDOC reconstitution and formation. This was confirmed by KEGG pathway modules from PICRUAST2.

The results of this paper demonstrate that microbes participate and dominate autochthonous DOC formation, especially in the downriver part of Lijiang River. Critical bacteria species were also identified, which is valuable for determining RDOC formation processes and the organic carbon sink mechanisms in karstic aquatic systems. However, the details of MDOC formation mechanisms remain unclear, as does the specific fraction of MDOC reconstituted from PDOC. Further efforts are required to clarify MDOC formation mechanisms and reconstitution ratios under conditions that have both high and low allochthonous SDOC fractions. Subsequently, this will enable the calculation of the quantity of autochthonous MDOC and the associated formational ratios.

CRediT authorship contribution statement

Qiufang He: Conceptualization; Data curation; Funding acquisition; Investigation; Visualization; Roles/Writing – original draft; Writing – review & editing.

Qiong Xiao: Data curation; Funding acquisition; Investigation.

Haijuan Zhao: Data curation; Formal analysis; Investigation; Methodology.

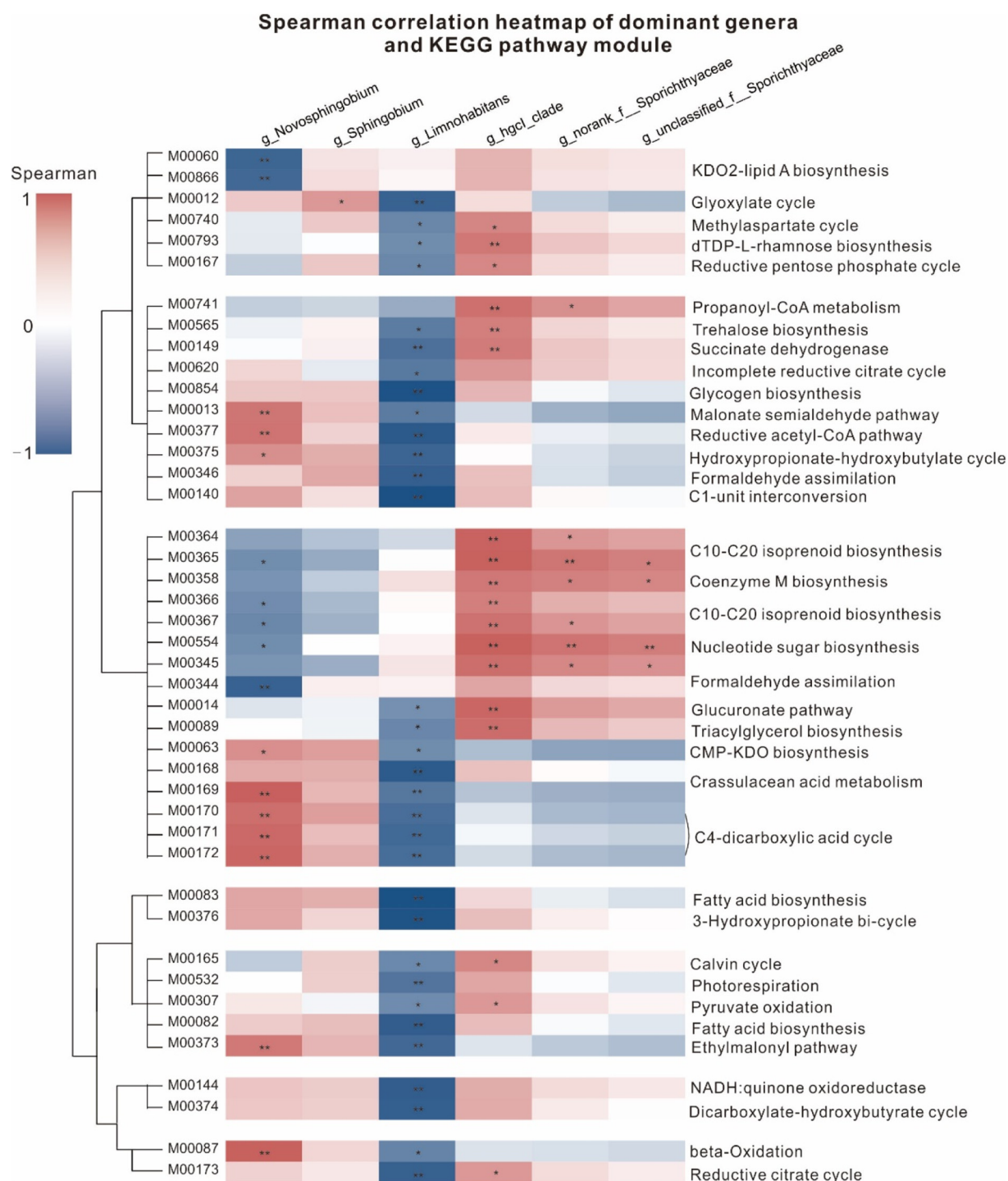


Fig. 8. Spearman's Rank correlation heatmap clusters of PICRUAST2 carbon synthesis KEGG pathway modules with the top six genera of the Lijiang River. Red and blue represent positive and negative correlations, respectively.

Min Cao: Formal analysis; Investigation; Writing – review & editing.
Cheng Zhang: Conceptualization; Funding acquisition; Project administration.
Yongjun Jiang: Conceptualization; Funding acquisition; Project administration; Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2021.152576>.

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