High stability of autochthonous dissolved organic matter in karst aquatic ecosystems: Evidence from fluorescence

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ABSTRACT

Biological carbon pump (BCP) in karst areas has received intensive attention for years due to their significant contribution to the global missing carbon sink. The stability of autochthonous dissolved organic matter (Auto-DOM) produced by BCP in karst aquatic ecosystems may play a critical role in the missing carbon sink. However, the source of dissolved organic matter (DOM) in inland waters and its consumption by planktonic bacteria have not been thoroughly examined. Recalcitrant dissolved organic matter (RDOM) may exist in karst aquatic ecosystem as in the ocean. Through the study of the chromophoric dissolved organic matter (CDOM) and the interaction between CDOM and the planktonic bacterial community under different land uses at the Shawan Karst Water-carbon Cycle Test Site, SW China, we found that C2, as the fluorescence component of Auto-DOM mineralised by planktonic bacteria, may have some of the characteristics of RDOM and is an important DOM source in karst aquatic ecosystems. The stability ratio (Fmax$_{(C2/(C1+C2))}$) of Auto-DOM reached $89.6 \pm 6.71\%$ in winter and $64.1 \pm 7.19\%$ in spring. Moreover, correlation-based network analysis determined that the planktonic bacterial communities were controlled by different fluorescence types of CDOM, of which C1 (fresh Auto-DOM), C3 (conventional allochthonous DOM (Allo-DOM)) and C4 (the Allo-DOM mineralised by bacteria) were clustered in one module together with prevalent organic-degrading planktonic bacteria; C2 was clustered in another tightly combined module, suggesting specific microbial utilization strategies for the C2 component. In addition, some important planktonic bacterium and functional genes (including chemotrophic heterotrophs and photosynthetic bacteria) were found to be affected by high Ca^{2+} and dissolved inorganic carbon (DIC) concentrations in karst aquatic ecosystems. Our research showed that Auto-DOM may be as an important carbon sink as the Allo-DOM in karst ecosystems, the former generally being neglected based on a posit that it is easily and first mineralized by planktonic bacteria.

1. Introduction

In recent years, much research has focused on the location of missing carbon sinks (Schindler, 1999; Ciais et al., 2013; Kirschbaum et al., 2019; Liu et al., 2021). An increasing number of studies have suggested that inland water play a significant role in the global carbon cycle and deserves further study (Cole et al., 2007; Liu et al., 2018, 2021). Recently, researchers have reported that in karst areas, aquatic
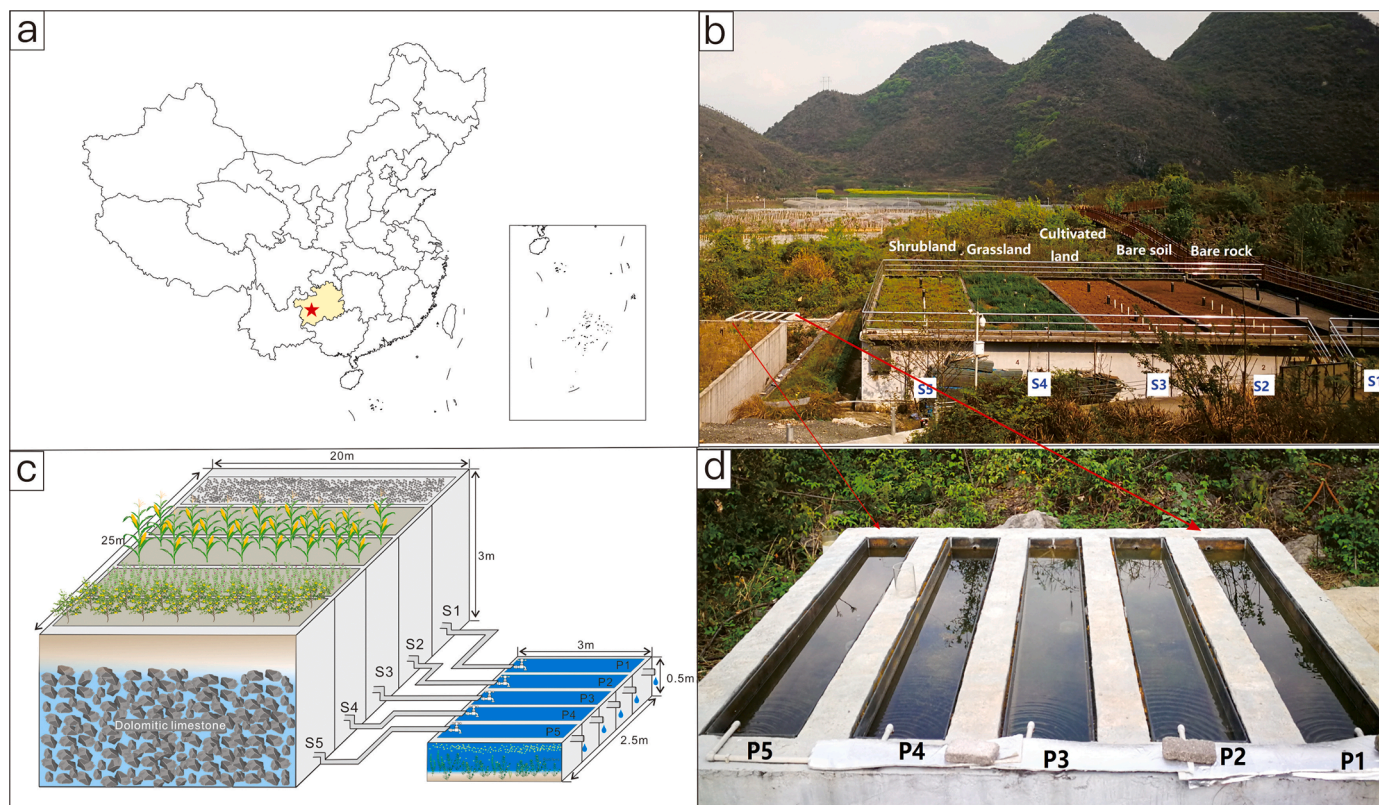
Fig. 1. (a) Location of the Shawan Karst Water-carbon Cycle Test Site; (b) The five tank watersheds with their different land uses; (c) Schematic diagram of test site (S and P represent spring and pond, respectively); (d) The five associated spring-fed ponds.

photosynthetic organisms can take up dissolved inorganic carbon (DIC), converting it into organic carbon and fixing it when they die. Biological remains are buried in reservoirs and lakes, creating a stable carbon sink in inland waters (Liu et al., 2010, 2011, 2018, 2021). The missing carbon sink associated with the strengthening of autochthonous production (AP) in inland ecosystems has been estimated to range from 0.38 to 1.8 Gt C yr^{-1} with large uncertainties (Liu et al., 2021). AP is an important carbon sink and dissolved organic carbon (DOC) accounts for more than 80% of the total organic carbon (TOC), especially in karst lake water columns, DOC is the dominant indicator of autochthonous productivity in karstic aquatic ecosystems (Noges et al., 2016; He et al., 2022), which is generally neglected in the calculation of the carbon budget based on the assumption that DOC is readily mineralised by planktonic bacteria (Sawakuchi et al., 2017; Drake et al., 2018; Ran et al., 2021). Therefore, it is crucial to study the stability of DOC in inland waters to assess missing global carbon sinks around the world. Based on the carbon isotope values, the high DIC concentrations promotes both phytoplankton and aquatic plant photosynthetic production. Photosynthetic autochthonous organic carbon contributes 60–80% of the total sediment and particulate organic carbon (POC) in karst aquatic ecosystems (Yang et al., 2016; He et al., 2020; Huang et al., 2020). He et al. (2021) applied the excitation-emission matrix (EEM) spectrum method and found that the DIC fertilization effect directly promoted autochthonous DOC (Auto-DOC) formation and indirectly promoted microbial-sourced DOC in the Lijiang River. These results confirmed the existence of a biological carbon pump (BCP) in karst areas. However, the stability of Auto-DOC in the water has not been explored, and it remains unclear what is the strategy of planktonic bacteria in the water column to consume Auto-DOC.

In aquatic ecosystems, DOM has been classified based on its origin (autochthonous or allochthonous) and bioavailability (labile or recalcitrant) (Muscarella et al., 2019). Much research has been conducted on allochthonous organic matter, and the parts that flow into lakes are

regarded as difficult to degrade. Recent reports have challenged this scenario by showing fast planktonic bacterial utilization of a highly labile pool of low molecular weight compounds of allochthonous origin in boreal streams (Ågren et al., 2008; Berggren et al., 2010), leading to the possibility that allochthonous DOC (Allo-DOC) could fuel carbon degradation by planktonic bacteria at different time scales (Holmes et al., 2008; Ward et al., 2013). The concentration of Auto-DOC in freshwater lakes depends mainly on aquatic primary productivity and the degradation of planktonic bacteria. Interactions between DOC and planktonic bacteria are central to biogeochemical cycles in aquatic ecosystems. Heterogeneous microorganisms in water bodies employ DOC as the main carbon source, while previous studies suggest that many primary production forms are preferentially degraded and eventually consumed by approximately 80% (Westrich and Berner, 1984; Cole et al., 1988; Guillemette et al., 2013, 2017). Previous studies have suggested that molecular weight, chemical composition, and elemental metrology are the basis for microbial consumption of organic carbon (Chrost et al., 1983; Amon and Benner, 1996). The organic carbon in algae is regarded as simple, labile, composed of small molecules, and easily consumed by planktonic bacteria (Chrost and Faust, 1983; Amon and Benner, 1996; Berggren et al., 2010; Berggren and del Giorgio, 2015), which may explain why the budget of Auto-DOC has been neglected in previous studies. However, studies have shown that the preferential breakdown and metabolites of Auto-DOC are resistant to microbial remineralization (Ogawa et al., 2001; Hach et al., 2020; DeFrancesco et al., 2021). The influence of Auto-DOC on planktonic bacterial communities has been demonstrated by incubating planktonic bacteria with different sources of DOC in laboratory studies (Perez and Sommaruga, 2006; Guillemette et al., 2013; Zhou et al., 2019, 2021a). The difficulty in distinguishing Auto-DOM and Allo-DOM in the complex field environment and the limitations of laboratory tests are challenges in the study of the utilization of DOC by planktonic bacteria in the field environments. Moreover, the disturbance of the phytoplankton

community structure caused by various complex environmental factors in natural lakes may distort the degradation dynamics between Auto-DOM and planktonic bacteria. Therefore, we required an ecosystem with clear sources of organic matter and relatively independent influencing factors, which would allow for an understanding of the degradation dynamics of autochthonous and allochthonous DOM by planktonic bacteria.

The key to solving the above problems is to distinguish the sources of chromophoric dissolved organic matter (CDOM) and construct a relationship between planktonic bacteria and each component. Many scholars have made great efforts to study the distinction between autochthonous and allochthonous sources of CDOM, but there are still many disputes. Recently, the combination of the EEM and fluorescence regional integration has been used to quantify the abundance and dynamics of DOM components in inland water (Yu et al., 2015; Zhao et al., 2017). Moreover, the rapid development of metagenomic technology has provided deep insight into the dynamics of planktonic bacterial communities in the field (Legendre and Gallagher, 2001). In particular, the emergence of network analysis methods has played a key role in explaining changes in lake microbial communities and environmental interactions (Eiler et al., 2012; Barberan et al., 2012; Kara et al., 2013). To date, researchers have focused on the interspecific interactions of lake communities but ignored the relationship between DOM and planktonic bacterial community metabolic activities as the carbon source and main nutrient. Understanding the relationship between DOM and planktonic bacterial community metabolic activities is crucial for understanding the consumption strategies of planktonic bacteria (Zhang et al., 2020).

Here, we present the results of planktonic bacterial changes and CDOM in four seasons under five different land uses at the Shawan Karst Water-carbon Cycle Test Site, SW China. The variation in the intensity of the CDOM fluorescence peak (Fmax) indicates the variation in the concentrations of the different components (Murphy et al., 2013). High-throughput sequencing of 16 s and parallel factor modeling (PARAFAC) of excitation-emission fluorescence spectroscopy were used to answer the following three questions: (1) How do seasonal changes affect the composition of autochthonous and allochthonous sources of CDOM in karst aquatic ecosystems under different land uses? (2) What are the effects of organic matter input from different proportions of autochthonous and allochthonous sources on the planktonic bacterial community structure? (3) How do DIC concentrations influence planktonic bacteria in relation to the carbon cycle under different land uses? We hypothesised that Auto-DOM formed through the microbial carbon pump (MCP) in karst aquatic ecosystems may be stabilised in certain environments.

2. Materials and methods

2.1. Description of the test site

The Shawan Karst Water-carbon Cycle Test Site (26°14–26°15 N, 105°42–105°43E) is located in Puding County, Guizhou Province, China (Fig. 1a). This region has a humid subtropical monsoon climate with an average annual precipitation of 1315 mm, approximately 80% of which occurs during the rainy season and an annual mean air temperature (T) of ~15.1 °C (Zhao et al., 2010).

We built five ecosystems with different land uses (Fig. 1b): bare rock (S1), bare soil (S2), cultivated land (S3), grassland (S4), and shrubland (S5). Each ecosystem was composed of a concrete tank that was 20 m long, 5 m wide, and 3 m deep and coated with epoxy resin to avoid the influence of concrete erosion on the tank hydrochemistry (Zeng et al., 2017). The bare rock tank was filled with dolomitic limestone gravel without soil. The bare soil tank was filled with soil and dolomitic limestone gravel but was not planted with any vegetation. The cultivated land tank was planted with corn from April to October, and a moderate amount of compound fertiliser was applied at the same time as

the seed in April. When corn died, it was buried *in situ*. In 2014, alfalfa and Roxburgh rose were planted in grassland and shrubland tanks, respectively, and were never harvested (Bao et al., 2021) (Fig. S1). There were side drainage holes in each tank feeding artificial ponds (P1-P5), which simulated natural karst springs (S1-S5) (Fig. 1c). Each pond and its associated spring system simulated a precisely known single land-use catchment of the same size (Zeng et al., 2017). In June 2019, *Chara spp.*, the dominant submerged plants in karst aquatic ecosystems, were transplanted into the ponds from a nearby river basin (Bao et al., 2020). More details on the tanks and ponds are provided in the Supporting Information.

2.2. Sample collection and monitoring of environmental parameters

Samples were collected in July and October 2020 and January and April 2021. A multiparameter water quality probe was used to measure T, pH, and dissolved oxygen (DO). DIC was titrated using an Aquamerck alkalinity test kit in the field with an estimated accuracy of 6 mg/L. The CO_2 (aq) concentration was calculated using Phreeqc (Zeebe and Wolf-Gladrow, 2001).

Five litres of water from each pond (mixed sample from the front area, middle area and back area of a pond) were stored in sterile polyethylene bottles and delivered to the laboratory on the same day. Two-litre water from each ecosystem were filtered through a 0.22 μm membrane and stored at −80 °C for Illumina MiSeq sequencing of 16 s rRNA. Each 1 L water was filtered through a 0.45 μm membrane and stored at −20 °C for the determination of chlorophyll a (Chl-a). Chl-a concentration was extracted using a 95% ethanol solution heated to 80 °C in triplicate (Sartory and Grobbelaar, 1984), and 150 mL of water from each pond was stored in brown glass bottles at 4 °C for later TOC determination. The TOC concentration was measured using an OI Analytical "TIC–TOC" Analyser.

2.3. CDOM fluorescence measurements

The details of the CDOM fluorescence measurements are provided in the Supporting Information. All the date were normalised to Raman units (R.U.) (Zhang et al., 2020). Using the alternating least-squares algorithm PARAFAC (Stedmon and Markager, 2005; Murphy et al., 2013), each EEM was divided into a series of three linear components and residual arrays. The EEMs of all samples ($n = 5$ ponds $\times$ 4 seasons) collected from the five ponds were included in the dataset modeled using PARAFAC and the drEEM toolbox in MATLAB R2017a. Split-half analysis and random initialization were used to validate the identified components. The maximum fluorescence intensity of each PARAFAC component (Fmax) and the percentage of each PARAFAC component in the water samples were calculated (Stedmon and Markager, 2005). The position of the PARAFAC component was determined using the Fmax value of the component and its Ex and Em wavelengths.

2.4. DNA extraction and bioinformatic analysis

Planktonic bacterial DNA was extracted with a DNA extraction kit for the corresponding sample. The concentration and purity were measured using NanoDrop One (Thermo Fisher Scientific, MA, USA). Primers 338F (5′-ACTCCTACGGGAGGCAGCA −3′) and 806R (5′-GGAC-TACHVGGGTWTCTAAT-3′) were used for PCR amplification (Zhang et al., 2020). PCR was performed in triplicate in 50 μL reactions. After sequencing on an Illumina Nova6000 platform, 250 bp paired-end reads were generated (Guangdong Magigene Biotechnology Co., Ltd., Guangzhou, China).

We divided the obtained downstream sequencing data into different samples by barcode sequence, and the barcode sequence was cut off. Primer sequences and distal bases were trimmed, and Cutadapt (v1.9.1) was used for quality filtering and removal of singletons. Potential chimaeric sequences were moved using Usearch (v8.1.1861), and cluster
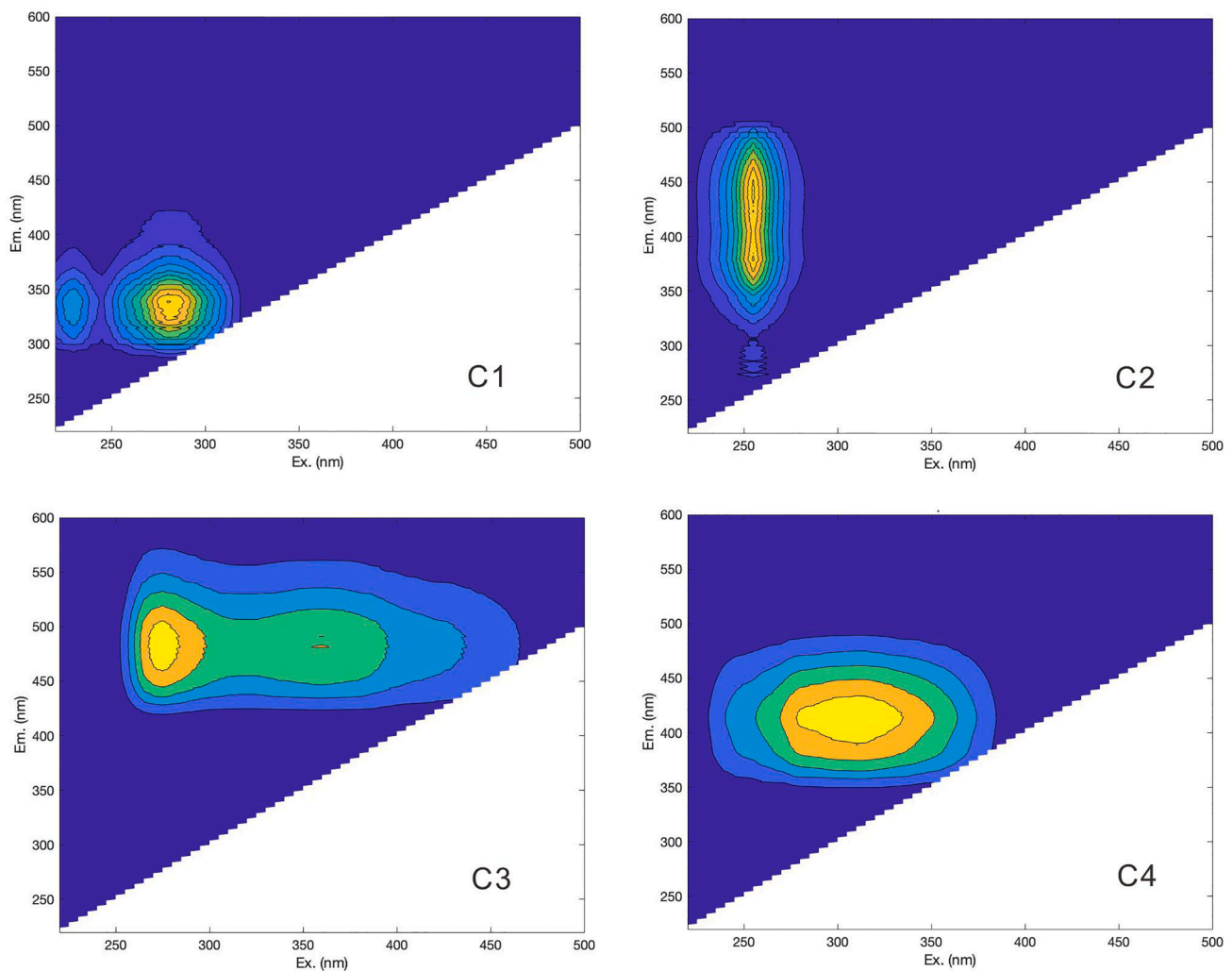
Fig. 2. Spectral shapes of the four fluorescent components identified by PARAFAC. C1 represents tyrosine-like (peak B) fluorophores, C2 represents low molecular humus-like (peak M) fluorophores, C3 represents allochthonous humus-like (peak C) fluorophores, and C4 represents microbial humus-like fluorophores.

analysis was performed on the processed sequences. OTUs were grouped by clustering sequences with 97% similarity levels (Edgar, 2010). OTU taxonomic data were obtained using Uclust (v1.2.22) to compare the representative sequences of OTUs with the Silva version 132 16S rRNA databases. Functional gene annotation was performed using functional annotation of the prokaryotic taxa (FAPROTAX) (Louca et al., 2017). The 16S rRNA gene sequences were deposited in the NCBI Sequence Read Archive (SRA) database under the number PRJNA810372.

2.5. Statistical analysis

The OTUs were combined with four CDOM and three hydrochemical parameter components to construct a network. The psych pair (R package) was used to calculate the Spearman correlation coefficient (p) between OTUs with a total of more than 96% and the Fmax of CDOM components in the sample and hydrochemical parameters. To reduce the complexity of the dataset, the relative abundance of OTUs >0.01% with significant correlations ($p < 0.05$) and robust correlations ($r > 0.6$) were chosen (Zhou et al., 2021b). Random networks with equal numbers of nodes and edges to real networks were generated based on Erdös–Rényi random networks (Erdös and Rényi, 2011). Gephi (v9.2) was used for attribute calculation of the network, module detection and visualization. We determined the connectivity of each node based on its within-module connectivity (Zi) and among-module connectivity (Pi) (Guimera and Amaral, 2005). Node topologies in the network were

classified into four categories: network hubs ($Zi > 2.5$ and $Pi > 0.62$), module hubs ($Zi > 2.5$ and $Pi < 0.62$), connectors ($Zi < 2.5$ and $Pi > 0.62$) and peripherals ($Zi < 2.5$ and $Pi < 0.62$) (Shi et al., 2016). The size of each node was proportional to the relative abundance of the planktonic bacterial community ($\log(n + 1)$). We converted the environmental variables by $\log_{10}(x + 1)$, except for pH, and conducted a collinearity analysis on the environmental factors to remove the high variance inflation factor (VIF) > 20 (Peng et al., 2021). The redundancy analysis (RDA) function was performed using Canoco5 software to explain the relationship between the composition of the microbial community and environmental factors (Oksanen et al., 2018). Spearman correlation coefficients between the four CDOM components and the relative abundance of the planktonic bacterial community were calculated using the SPSS software. Mantel tests were performed to compare environmental factors, abundant genera and keystone taxa.

3. Results

3.1. Physiochemical properties of the five different ponds

The physiochemical parameters of the water in the five ponds (P1-P5) showed obvious seasonal variations (Fig. S2). Water pH ranged from 8.09 to 9.29. The HCO_3^- concentrations ranged from 1.2 mmol/L to 3.9 mmol/L. High concentrations of HCO_3^- occurred in P4 and P5. In contrast, low HCO_3^- concentrations were observed in P1 and P2. The
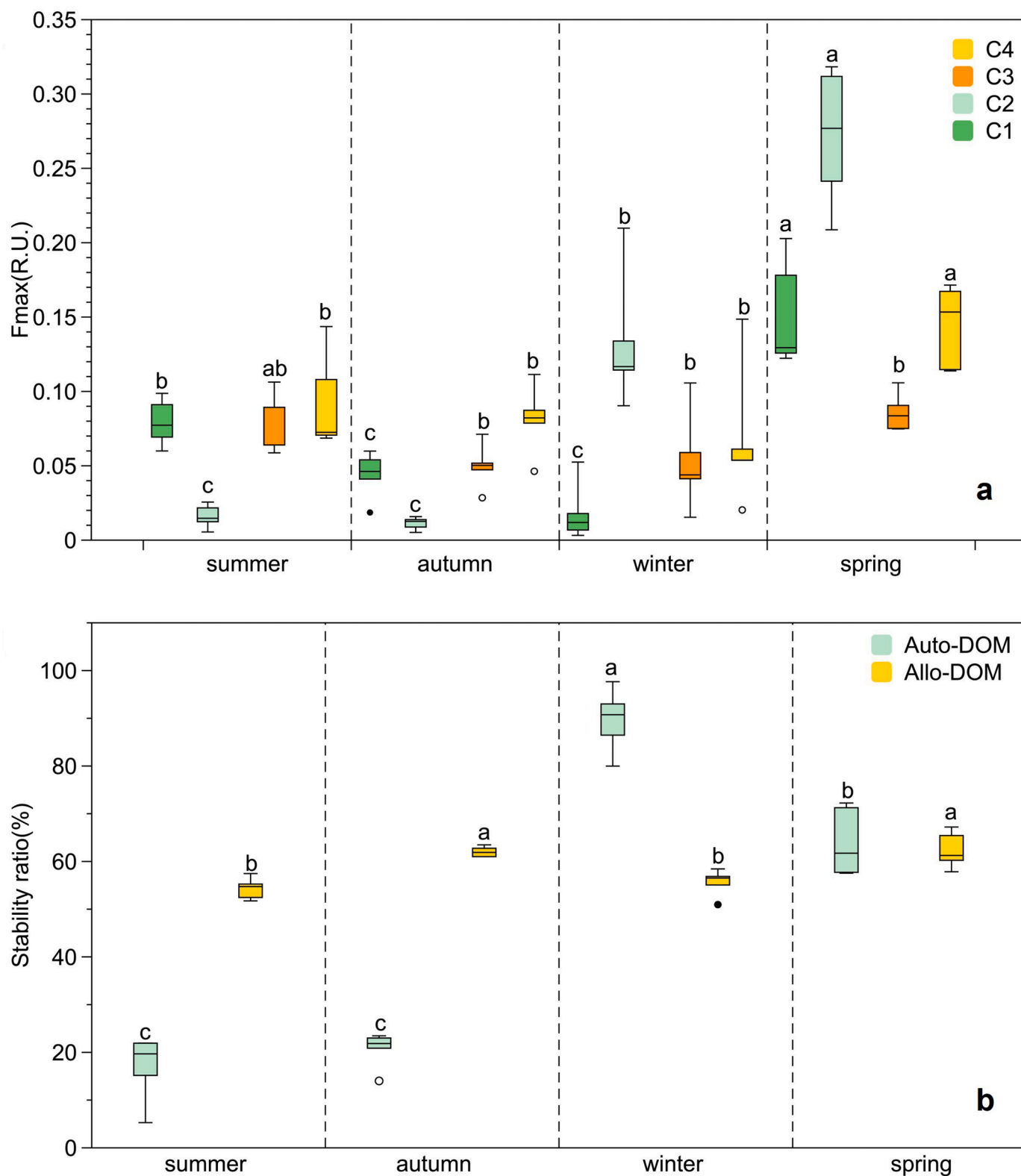
Fig. 3. (a) Seasonal variations of four components in five ponds. (b) Proportion of Auto-DOM after mineralization $Fmax_{(C2/(C1+C2))}$ and proportion of Allo-DOM after mineralization $Fmax_{(C4/(C3+C4))}$. The letters above the bars (in a, b, c) represent the differences of significant among the four seasons ($p < 0.05$).

ponds were characterised by high saturated concentrations of DO in spring, summer and autumn (130–235%), but low values in winter. Chl-a generally showed temporal and spatial dynamics similar to DO, with the maximum in P4 and P5 (4.94 µg/L and 3.07 µg/L in summer and 2.04 µg/L and 1.63 µg/L in spring, respectively). Unlike Chl-a and DO,

there were steady variations in TOC over the first three seasons and increases in spring.

3.2. Changes in CDOM compositions in different pond waters

Four distinct components were identified (Fig. S3) with ex|em wavelengths of 230 (280)|326 nm, 255|381(444) nm, 275(340)|466 nm and 310|412 nm. Component C1 represents tyrosine-like (peak B) fluorophores (Zhou et al., 2019). C2, C3, and C4 showed analogous positioning to different humus -like fluorophores; C2 was categorised as low molecular humus -like (peak M) fluorophores associated with autochthonous production (Coble 1996; Fellman et al., 2010; Mudarra et al., 2011; McIntyre and Gueguen, 2013), C3 represented allochthonous humus-like (peak C) fluorophores with a high molecular weight and aromatic organic compounds (Amaral et al., 2021), and C4 was categorised as microbial humus-like fluorophores (peak M) (Wang et al., 2012; Hambly et al., 2015; Amaral et al., 2020) (Fig. 2).

The tyrosine-like component, C1, represented amino acids derived from plankton. The Fmax of C1 was low in winter (0.018 ± 0.0198 R. U.), but high in spring (0.152 ± 0.0365 R.U.) (Fig. 3a). The low molecular component C2, which was humus-like, changed greatly from summer and autumn (0.014 ± 0.0064 R.U.) to winter and spring (0.202 ± 0.0466 R.U.) and was the dominated fluorescence component in winter and spring (Fig. 3a). The Fmax values of components C1 and C2 showed a trend similar to that of the TOC. C3, which represented allochthonous humus-like features, showed a slight change over the four seasons. Microbial humus-like C4, was the dominated fluorescence component in summer and autumn, accounted for 39.16 ± 5.99% of all components in the five ponds and halved in winter and spring to 22.50 ± 3.61%.

Stability refers to the resistance to degradation of microorganisms. Biosynthetic compounds not modified by secondary abiotic reactions are presumably to be resistant to degradation on the long term (Benner et al., 1992; Dittmar, 2015). The cellular and degradation products of microbes can be selectively preserved due to their chemical structure and adsorption to mineral surfaces (Sollins et al., 1996; von Lützow et al., 2006; Liang et al., 2017). We defined the stability ratio as the proportion of mineralised organic matter in the total organic matter Fmax of each source (autochthonous or allochthonous). C2 and C4 represent the components of organic matter from autochthonous and allochthonous sources, respectively, after mineralization by microorganisms. We can summarise these as the stable components of the two sources. For example, the stability ratio of Auto-DOM was $Fmax_{(C2/(C1+C2))}$ and that of Allo-DOM was $Fmax_{(C4/(C3+C4))}$. Fig. 3b shows that the stability ratio of Auto-DOM was low in summer (16.8 ± 7.00%) and autumn (20.6 ± 3.84%) and high in winter (89.6 ± 6.71%) and spring (64.1 ± 7.19%). The stability of the two components from the same source is different. The sources of the two Auto-DOM are mixed. The fresh Auto-DOM, such as tryptophan, is easily degraded, which makes Auto-DOM seeming to be labile. The stability ratio, therefore, should be used to quantify the stability of different sources to obtain a clearer understanding of the importance of Auto-DOM in carbon sinks. Auto-DOM had two different phases throughout the year: summer to autumn and winter to spring. However, the stability ratio of the Allo-DOM was the same throughout the year.

3.3. Composition of planktonic bacterial communities

In total, 47,991 clean 16S rRNA gene sequences and 5154 OTUs were obtained during macroalgal degradation. The coverage of each sample was greater than 0.97. These OTUs (at a 3% dissimilarity threshold) were classified into 43 phyla, 113 classes, 326 orders, 619 families and 1074 genera. Owing to the high proportion of *Proteobacteria* at the phylum level (Fig. S4a), to facilitate our in-depth analysis, we divided it into three subgroups *Alphaproteobacteria, Gammaproteobacteria*, and *Deltaproteobacteria*, at the phylum level.

A total of 25 major phyla (>0.01) were detected in the samples from different ponds during the four seasons (Fig. S4a). *Proteobacteria (Alphaproteobacteria* and *Gammaproteobacteria), Actinobacteria* and *Bacteroidetes* were the dominant phyla, while *Actinobacteria* disappeared in winter. In summer, *Alphaproteobacteria* were rare in P4 and P5, whereas *Bacteroidetes* were abundant in P3 and P4. The community structure of the five ponds in winter was similar to that in summer. However, the dominance of *Bacteroides* in P3 and P4 decreased significantly, whereas *Actinobacteria* abundance increased. *Gammaproteobacteria* dominated the five ponds during the winter. In spring, the changes among the five ponds were different from those in other seasons, especially in P4 and P5. *Limnohabitans, Microbacteriaceae_f, Polynucleobacter, hgcl_clade*, and *Sediminibacterium* were enriched in the ponds (67.3%) (Fig. S4b). Except in spring, *Polynucleobacter* were generally prevalent in P5 (36.4% in summer, 29.5% in autumn, and 45.2% in winter), and a higher abundance appeared only in P4 in spring. *Sphingopyxis* decreased in P4 and P5, except in spring, whereas it increased in P4 in spring. The abundance of the *hgcl_clade* increased extremely in spring.

4. Discussion

4.1. Effects of DIC fertilization on spatiotemporal changes in fluorescence components

The HCO_3^- concentrations showed large spatiotemporal variations in the five ponds due to different seasons and land uses. In our experiment, the major factor affecting HCO_3^- concentrations was the influence of spring water input caused by changes in soil respiration under different vegetation coverage conditions (Zeng et al., 2019). P4 reflected the influence of grassland with more litter, more developed roots, more soil microbes, and the largest soil respiration in the alfalfa planting period, resulting in the highest HCO_3^- concentrations of S4 in spring water. P3 and P5 had less development of plants than P4, thus, their HCO_3^- concentrations were lower. The higher DO concentrations in P4 and P5 during the growing season, combined with the higher HCO_3^- concentrations indicated a more intense BCP in the two ponds (Bao et al., 2020). This was in agreement with previous research showing that hydrochemistry under different land uses may influence the primary productivity in the corresponding surface waters (Hayes et al., 2015; Bao et al., 2020).

Organic matter produced by primary production could be accompanied by photodegradation and biodegradation, and its composition could change. We found that the Fmax of C1 and C2, which represented autochthonous organic matter, were higher in P4 and P5 (Fig. S5). Previous studies have shown that DIC fertilization affects aquatic photosynthesis in pond ecosystems (Zeng et al., 2019). Therefore, the higher Fmax of C1 in P4, which represents fresh Auto-DOM, may have been due to DIC fertilization. Simultaneously, C2 had the highest Fmax in water. He et al. (2021) found that C2 is positively correlated with DIC in the Lijiang River. We speculated that, in addition to DIC fertilisation, the stability of DOM at high DIC levels may be another cause. A previous study at the site (Bao et al., 2020) showed that *diatoms* were the dominant algae in P4 and were more conducive to preservation as they had more stable components than other algae (Landa et al., 2014).

To explore the characteristics of organic matter from different sources, we analysed the contribution rate and stability ratio of Auto-DOM and found that from the perspective of the contribution rate, the total Fmax values of the four components were almost the same in summer, autumn, and winter, but increased significantly in spring, which was consistent with the change in TOC. This was mainly due to the increase in autochthonous components during winter and spring (Fig. 3a). The proportion of the C2 component in winter and spring was approximately 47.1 ± 9.60%, a significant proportion of inland water. Previous studies have shown that the DOM decomposition rate, in theory, could be a function of its concentration (Dittmar et al., 2015), and the high Fmax of C2 in these two seasons not only indicates a high concentration but also explains the resistance of C2 to microbial degradation. The release of autochthonous components from planktonic and submerged ecosystems began in November, and Auto-DOM,
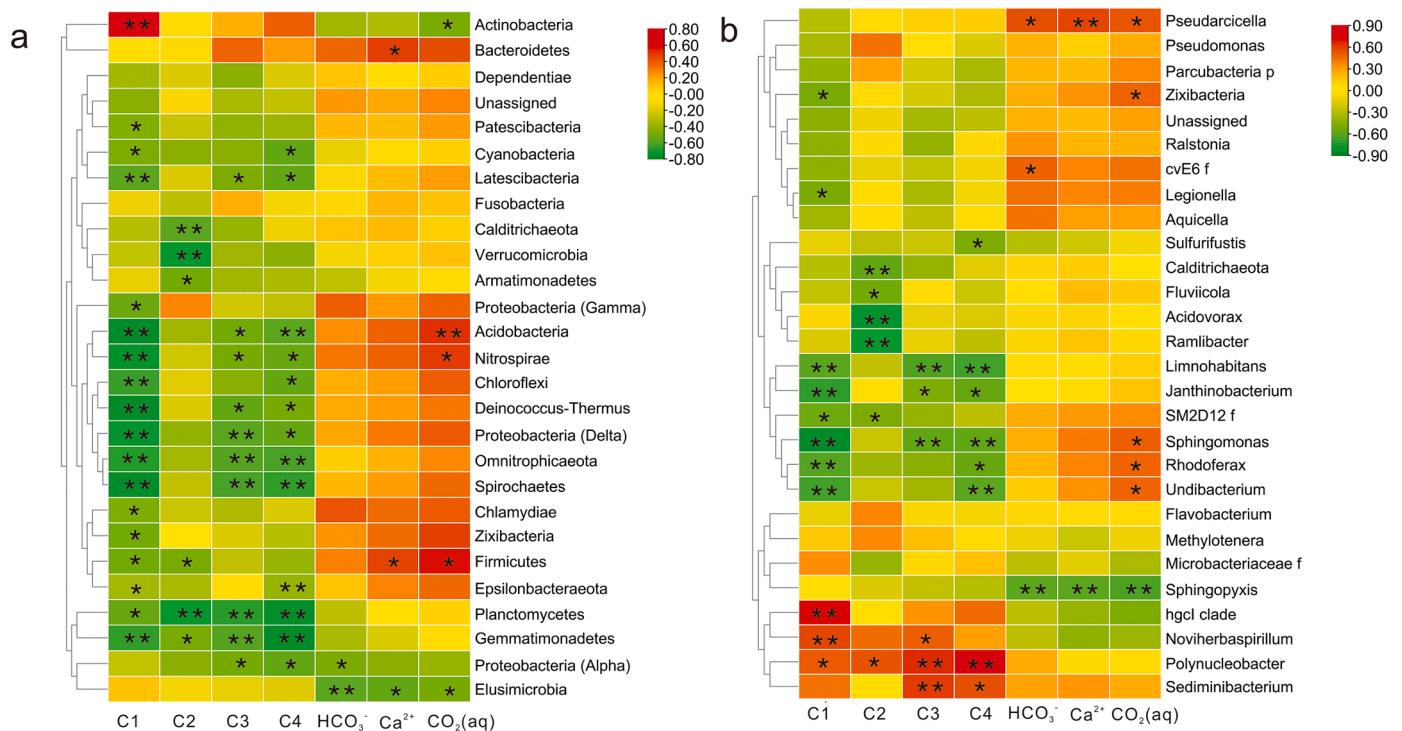
Fig. 4. The RDA plots of planktonic bacterial community and environmental factors at different classification levels. (a) phylum level; and (b) genus level. Red arrows: environmental factors, Blue arrows: planktonic bacterial community.

especially C2, was not the highest in summer. With the increase in rainfall in summer, the C2 components released by the decay of algae in the previous year were removed by water flow, whereas the newly formed planktonic and submerged components were still in the growing season. Only a small portion of the C2 components was released, and the high deposition flux in summer also resulted in more fixed organic matter settling into the sediment in the form of POC. These results coincide with the fact that planktonic bacteria may produce humus-like substances in lakes after modifying protein-like substances, thereby increasing the proportion of humus-like substances and decreasing the proportion of protein-like substances (Hanamachi et al., 2008). This has been proven in incubation experiments with samples from the Tibetan Plateau and the Antarctic region (Zhou et al., 2019). The stability ratio indicated that Auto-DOM was preserved in the form of C2. The stability of Allo-DOM (C4) has been proven in previous studies (Guillemette et al., 2013, 2017; Zhou et al., 2021a), which suggests that the stability ratio defined is meaningful from another perspective. Allo-DOM is the result of multiple mineralizations by microorganisms in soil and spring water, and it is more stable than fresh organic matter formed in lakes. Stability ratio in our research is therefore a relative concept since the "dilution limits" and "recalcitrant mechanism" are still an important debate question to research in the ocean (Jiao et al., 2010; Arrieta et al., 2015). The organic matter produced by aquatic plants may be used by planktonic bacteria, similar to terrestrial plants, and the recalcitrant humus is eventually buried and preserved. Thus, this part of organic matter should not be ignored when discussing the contribution of inland lakes to global carbon sinks. The importance of autochthonous production in inland lake carbon sinks could be neglected if one compares mineralised Allo-DOM with fresh Auto-DOM, rather than compare the stability of the metabolic products of both.

There have been many experiments on bacterial degradation of phytoplankton, both in ocean and inland waters. Hanamachi et al. (2008) found that although the phytoplankton-produced organic matter is readily used by planktonic bacteria quickly, molecular rearrangement by planktonic bacteria is an important factor in Auto-DOM burial. In addition, the algal organic groups were divided into neutral aldoses, amino acids, fatty acids. The organic composition of the labile fraction of phytoplanktonic organic matter was found to vary depending on the phytoplankton groups. *Diatoms* have a more recalcitrant organic

component, glycine (Hecky et al., 1973). *Diatoms* are a class of phytoplankton sensitive to carbon. High DIC and Ca^{2+} levels are important characteristics of karst aquatic ecosystems. Higher CO_2 concentrations lead *diatoms* to increase in abundance (Tortell et al., 2002; Low-Décarie., 2015), allowing *diatoms* to dominate phytoplankton communities in this aquatic ecosystem.

4.2. Effects of CDOM components and hydrochemical properties on the composition of planktonic bacteria

We demonstrated that different CDOM components and physicochemical properties were crucial drivers of planktonic bacterial composition in the five ponds. The relationship between planktonic bacteria and environmental factors was determined by RDA at different classification levels. At the phylum level (Fig. 4a), T, pH, and C1 ($P < 0.05$) played a vital role in structuring the abundant bacterial compositions, and T was the dominant environmental factor affecting the planktonic bacterial community, which was mainly reflected in the seasons. HCO_3^- and humus-like C3 also played a role in some planktonic bacterial communities.

At the genus level (Fig. 4b), T, Chl-a, C1, and C4 were the main factors affecting the planktonic bacterial compositions ($P < 0.05$). This relationship suggests that humus-like components may also influenced planktonic bacterial communities, which differs from the results of previous studies on the Qingliu River (Zhang et al., 2020). This was because our research area showed little anthropogenic pollution, while previous research focused mainly on rivers flowing through the cities and industrial zones. Exogenous pollutants would greatly cover the influence of Auto-DOM and Allo-DOM on the microbial community, which would impede the exploration of the influence of DOM on planktonic bacteria. The clear boundaries of our study area make it clear that the planktonic bacterial community was affected by the karst environment and allowed us to determine the organic matter utilization strategy of planktonic bacteria. In addition, we found that C1 had a significant effect on the community structure, but C2 did not show this effect. These results suggest that, in addition to T and pH, both Auto-DOM and Allo-DOM can affect planktonic bacterial communities in natural karst aquatic ecosystems. However, the DOM components after mineralization may have no significant effect on planktonic bacteria due to their

Fig. 5. Heatmap showing the relationship of physiochemical properties and CDOM components with the bacterial communities at the phylum level (a) and genus level (b). *$p < 0.05$, **$p < 0.01$.

recalcitrant nature.

Through the above analysis, we constructed Spearman correlations between the percentages of the four CDOM components and the concentrations of HCO_3^-, CO_2(aq), and Ca^{2+} and the relative abundance of planktonic bacteria in the five ponds. These results suggest that tryptophan-like C1 and humus-like C3 and C4 have synergistic effects on most planktonic bacteria. At the phylum level, most planktonic bacteria were simultaneously negatively correlated with the four components (Fig. 5a) ($P < 0.05$). The phylum *Actinobacteria* was significantly positively correlated with C1 ($P < 0.01$), whereas *Gammaproteobacteria* was negatively correlated with C1 ($P < 0.05$). The former is considered competitive under resource-poor and aromatic-rich conditions (Li et al., 2020), and the latter responds to the enrichment of *diatom*-derived DOM (Landa et al., 2014) and utilises proteins, amino acids, and extracellular polysaccharides (Blanchet et al., 2016; Sun et al., 2018; Zhao et al., 2019). The relationship between the two phyla and C1 illustrates the competitive relationship between them, which is consistent with the results obtained in previous laboratory degradation experiments (Zhou et al., 2021a). In addition, the significant negative relationship between CO_2(aq) and *Actinobacteria* indicated that the competitive advantage of this phylum would gradually decreased in water with higher CO_2(aq). *Bacteroidetes* are capable of decomposing carbohydrates, starch polysaccharides, and hydrolytic cellulose (Bauer et al., 2006). Although no significant correlation was found between *Bacteroidetes* and the related components in our study, the extremely significant positive correlation between *Bacteroidetes* and Ca^{2+} indicates that chemotrophic heterotrophic planktonic bacteria occupy a dominant position in a high Ca^{2+} environment. *Alphaproteobacteria*, the dominant planktonic bacteria in the community (Fig. S4a), was negatively related to humus-like C3, C4 and HCO_3^- ($P < 0.05$) and was competitive under resource-poor and aromatic-rich conditions. Low TOC concentrations characterise all ponds, with the mean value of 1.35 mg/L, and the maximum of 7.79 mg/L occurring in spring. In such a low-resource environment, although *Alphaproteobacteria* can become the most abundant planktonic bacteria, there is still a negative correlation between *Alphaproteobacteria* and the two allochthonous components. This was

contrary to the results of the research on the response of planktonic bacteria in composition and function to the various DOM in the Qinhuai River (Zhang et al., 2020), as the newly imported unstable DOM would be consumed quickly. Notably, the relationship between the C2 and planktonic bacteria was significantly different from that of the other components. In particular, no correlation was observed between the C2 and several key chemoheterotrophic planktonic bacteria.

At the genus level, our research showed that there was no preference between humus-like and tryptophan-like component consumption in the genus *Polynucleobacter* (Fig. 5b). This is inconsistent with the previous studies showing that *Polynucleobacter* prefers allochthonous humus in aquatic ecosystems (Steinberg, 2014; Adesanoye, 2015; Zhang et al., 2020). There was a strong relationship between the *hgcl_clade* and C1, which mainly degrades *cyanobacteria*. The *hgcl_clade* was negatively correlated with CO_2(aq), indicating that there were fewer *cyanobacteria* in the high CO_2(aq) environment, which is consistent with the conclusion found at the same research site that *diatoms* and *Chlorophyta* were more suitable for growth than *cyanobacteria* under a high DIC environment and appropriate nitrogen and phosphorus conditions (Bao et al., 2021). The abundant genera *Limnohabitans* and *Sphingomonas* were significantly related to C1, C3, and C4 because *Limnohabitans* can utilise substrates produced by different algae (Steinberg, 2014; Adesanoye, 2015; Karel et al., 2020). *Sphingomonas* is capable of energy metabolism and xenobiotic biodegradation metabolism (Li et al., 2020). The relationship between the CDOM components and these planktonic bacteria was negative because the poor resource environment, as mentioned above, led to insufficient organic matter. At the same time, in addition to *Polynucleobacter*, we found that the above abundant genera associated with organic matter degradation had no relationship with C2. This further confirmed the specific planktonic bacterial utilization characteristics of this component.

4.3. Carbon dominates the co-occurrence network of planktonic bacteria in the five ponds

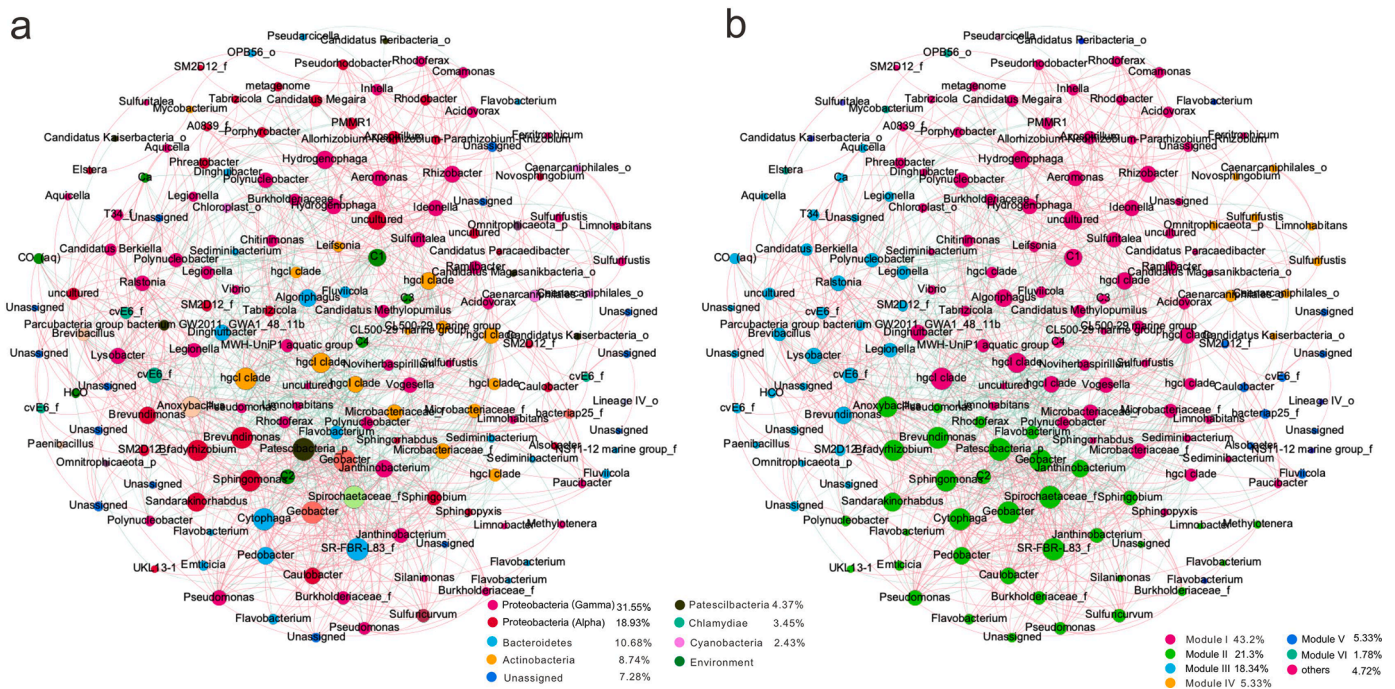To examine the interrelationship between selected OTUs with CDOM

Fig. 6. Co-occurrance network analysis between OTUs and CDOM components and physiochemical variables in the five ponds. The node size is proportional to the connections number, and the nodes are coloured on the basis of phylum (a) and module (b). The pink and green lines represent positive and negative relationships, respectively.

components and three hydrochemical indices, a co-occurrence network of five ponds in four seasons was constructed based on Spearman's correlations between OTUs with the four CDOM components and three hydrochemical indices. Thus, we elucidated the interactions among key planktonic bacterial communities from a new perspective (Fig. 6). According to our results, 1252 edges were captured among 169 nodes, indicating a significant interrelation between the abundant species. The network diameter, average path length, average clustering coefficient, and modularity of the real network were higher than those in the Erdös–Rényi random network (Table S1). The nodes were divided into 17 planktonic phyla. *Proteobacteria* (including *Gammaproteobacteria* and *Alphaproteobacteria*), *Bacteroidetes, Actinobacteria* and *Patescilbacteria* accounted for 72.18%. The abundant genera *hgcl_clade, Polynucleobacter, Flavobacterium, Sphingomonas, and Janthinobacterium* exhibited strong connections.

The nodes were divided into 13 modules, among which six modules had a strong correlation. Tyrosine-like C1 and the humus-like C3 and C4 were found in Modules II and C2 was in Module I. Hydrochemical indices, such as Ca^{2+}, HCO_3^- and $CO_2(aq)$, were aggregated in Module III. The species in Module I primarily belonged to *Gammaproteobacteria* and *Actinobacteria*. The nodes in Module II showed the highest average degree and closeness centralities (Fig. S6), indicating a stronger community relationship, and mainly included *Gammaproteobacteria, Bacteroidetes, Deltaproteobacteria* and *Alphaproteobacteria*. Generally, the degradation of organic matter and the adaptation of planktonic bacteria to the environment is a community behavior (Berry and Widder, 2014). Therefore, using the co-occurrence diagram, we can more clearly explain the characteristics of the consumption of organic matter by planktonic bacteria and the influence of environmental changes. Seventeen genera, including *hgcI_clade, Flavobacterium, Polynucleobacter, Burkholderiaceae, Hydrogenophag, Chloroplast*, and *Legionella* (module hubs and connectors) were the keystone taxa in our network (Fig. S7a). No network hubs (Pi > 0.62 and Zi > 2.5) were detected in the network. Most keystone taxa were rare planktonic bacteria, which was in agreement with previous studies (Shi et al., 2016). The disappearance of keystone taxa in certain environments causes the disassembly of

modules and networks, but the importance of keystone taxa play depends on the environmental changes (Lupatini et al., 2014). To further clarify the influence of environmental factors on the microbial communities and keystone taxa, we conducted a Mantel test (Fig. S7b). The abundance of the genera was significantly correlated with T, C1, C3, and C4 ($P < 0.01$), and the relationship with C2 was weaker ($P < 0.05$). Only T, C1, and C3 had a significant relationship with keystone taxa ($P < 0.05$). Overall, this is consistent with previous analyses showing that organic matter and T dominate planktonic bacterial community changes.

Our research shows that in such a karst aquatic ecosystem, without the interference of anthropogenic factors, differences in the CDOM components play a significant role in the construction of the planktonic bacterial community. The figure indicates that planktonic bacteria seem to have had a similar relationship with autochthonous and allochthonous DOM; two terrestrial components, C3 and C4, and an autochthonous component C1 are clustered in Module I, and some planktonic bacteria were associated with carbon (C) and nitrogen (N) cycles. For instance, the *hgcl_clade*, which is dominant in this module, plays a crucial role in *cyanobacteria* degradation, and *Limnohabitans* play a prominent role in the metabolism of organic matter in aquatic ecosystems (Kasalicky et al., 2013). From the results of the correlation analysis, we suggest that the autochthonous fresh component and two allochthonous components were the main carbon sources for heterotrophic planktonic bacteria, whereas the traditional view is that allochthonous components in aquatic ecosystems are relatively stable (Guillemette et al., 2013). Many recent studies show that although old carbon in soil is considered stable, it becomes more biolabile once it enters the aquatic ecosystem, known as "priming" (Marın-Spiotta et al., 2014). Due to the soil's physical structure, mineral adsorption and bioenergy constraints on soil bacterial communities may more critical in stabilising and storing organic matter than previously thought (Kemmitt et al., 2008; Schmidt et al., 2011). Therefore, when Allo-DOM leaves the soil environment and there are no original "partial refuges" to keep it temporarily stable, the priming effect from fresh organic matter entering the water body increase degradation. Notably, humus-like C2 was clustered in Module II.
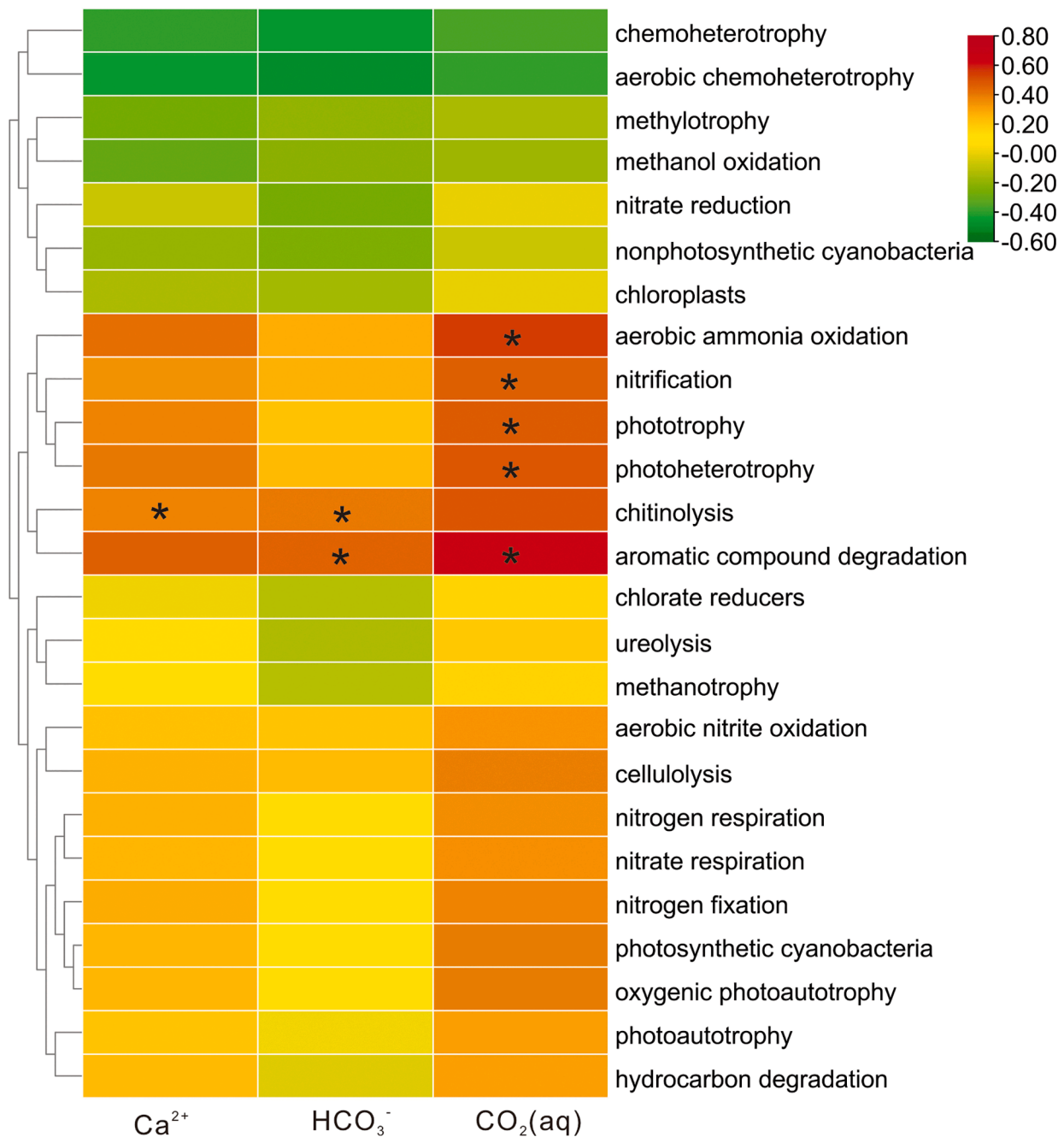
Fig. 7. Spearman's correlation-based functional profiles predicted by FAPROTAX for the planktonic bacteria and three hydrochemical indices in the five ponds. *p < 0.05.

The closer relationship may be due to the recalcitrance of Auto-DOM after microbial mineralization, which requires the cooperation of planktonic bacterial communities. Planktonic bacteria may alter the molecular structure of DOM during degradation, making DOM resistant to further mineralization, thereby preserving carbon (Ogawa et al., 2001; Smith et al., 2017). C2 is the transformation of labile C into stabilised forms by planktonic bacteria. On this basis, we speculate that C2 may be a component of recalcitrant dissolved organic matter (RDOM) in inland water similar to that generated by the MCP mechanism proposed in the ocean and soil (Jiao et al., 2010; Liang et al., 2017). Although the reasons behind the long-term stability of DOM remain unknown, three important hypothesises have been shown: "environment hypothesis", "intrinsic stability hypothesis", and "molecular diversity hypothesis" (Dittmar, 2015). The nutritional state of the environment in our study

was suitable for the survival of planktonic bacteria. Therefore, the weak correlation between some key chemotrophic heterotrophs and C2, indicates that C2 may not be utilised by them. C2 was a recalcitrant humus component. All characteristics are consistent with the first two hypotheses and indicated that C2 may be a type of RDOM in inland waters.

4.4. Prediction of bacterial function genes and their relationship with Ca^{2+} and DIC

The functional genes for aerobic chemoheterotrophy, phototrophy, photoheterotrophy, nitrate reduction, methylotrophy, and methanol oxidation were the core genes for the C cycle in the five ponds (Table S2). To explore the specificity of these genes in karst areas, we constructed relationships between the functional genes and

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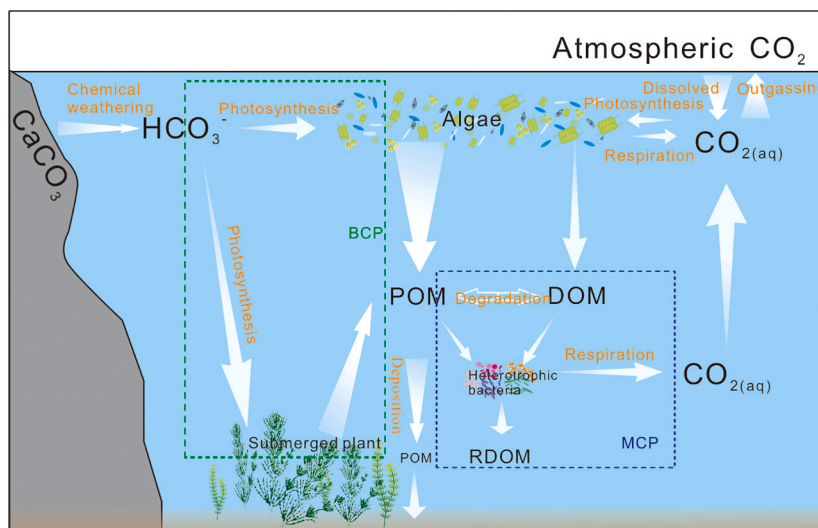
Fig. 8. Conceptual model diagram showing the carbon sequestration process in a karst aquatic ecosystem. The RDOM in the figure represents the C2 in the paper.

hydrochemical indices (Fig. 7). Functional genes for aerobic chemo-heterotrophy showed a significant negative correlation with HCO_3^-. HCO_3^- was positively correlated with chitinolysis. Ca^{2+}, HCO_3^-, and $CO_2(aq)$ were positively correlated with the functional genes involved in aromatic compound degradation. In addition, the functional genes for phototrophy, photoheterotrophy, and nitrification had a positive relationship with $CO_2(aq)$.

Previous studies have found that elevated atmospheric CO_2 concentrations may affect planktonic bacterial communities with regard to C assimilation and decomposition (He et al., 2010; Man et al., 2020). Karst aquatic ecosystems are characterised by high Ca^{2+} and DIC concentrations, and elevated atmospheric CO_2 concentrations also lead to an increase in DIC in water. An environment with high DIC concentrations can promote more intense photosynthetic production. Chemo-heterotrophy, based on the dominant functional gene predicted by FAPROTAX, was significantly reduced in the high DIC environment, suggesting that compared with non-karst areas, there were fewer che-moheterotrophic planktonic bacteria. This may promote the preservation of autochthonous organic carbon in karst areas. Moreover, the positive correlation between chitinolysis and aromatic compound degradation genes and the three hydrochemical indices suggests a higher abundance of planktonic bacteria related to the degradation of aromatic and chitin compounds in a high DIC environment. In conclusion, the high Ca^{2+} and DIC environments in karst areas have a significant impact on the planktonic bacterial communities and the associated carbon sequestration and organic matter degradation. This kind of environment favors a strong BCP effect generating more Auto-DOM, which initiates the priming effect and leads to the degradation of allochthonous organic matter.

5. Conclusion

We investigated the variation in the CDOM components of five simulated aquatic ecosystems under different land uses with relatively independent influencing factors during four seasons. Our results showed that Auto-DOM had significant seasonal variation, characterised by two main stages: the summer-autumn stage and the winter-spring stage. The Auto-DOM (C1+C2) accounted for $33.0 \pm 6.33\%$ in the first stage and $61.6 \pm 6.99\%$ in the latter stage. This indicated that seasonal factors must be considered when determining the contribution of Auto-DOM in karst aquatic ecosystems. C2, which is mineralised by bacteria, maintained a high level of contribution in the winter-spring stage, suggesting the high stability of it and leads to high stability ratio of Auto-DOM.

Based on the investigation of planktonic bacterial community and

the relationships between CDOM and planktonic bacteria, in combination with the analysis of microbial functional genes, we found that after planktonic bacterial mineralization, C2 may become more recalcitrant than C1 originating from phytoplankton. The stability of C2 was confirmed from a microbial perspective. Moreover, the correlation analysis of hydrochemical indices, Ca^{2+}, HCO_3^-, and $CO_2(aq)$, with planktonic bacterial communities and functional genes showed that the karst aquatic ecosystem had a significant impact on some important bacteria related to photosynthesis and chemoautotrophy, which may explain the relative stability of Auto-DOM in karst aquatic ecosystems. Based on these characteristics, we suggest that C2 may be an important RDOM in karst aquatic ecosystems (Fig. 8). We need to further test this conclusion through laboratory culture studies in the future. More importantly, this study provides new insights into the important contribution of inland lakes to the global carbon sink. In addition, we should strengthen research on the contribution of related Auto-DOM in lake sediments to the global carbon cycle.

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.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2022.118723.

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