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Plankton distribution patterns and the indicative significance of diverse cave wetlands in subtropical karst basin

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In subtropical karst area, small wetlands are widely distributed owing to the well-developed karst and the high degree of groundwater-surface water interaction. However, detailed plankton community composition, spatial-temporal dynamics, and its assembly mechanism were rarely discussed. To address these, plankton distribution patterns and its relationship with hydrological conditions and environment gradients were analyzed to discover the biological characteristics of wetlands in a typical karst basin in South China. The results showed that the wetlands were diverse in types due to the hydrogeological background. Water quality evaluation according to major ions analyses showed good results, with significant differences among samples and a poor status when measured by biotic indicator such as *Escherichia coli*. During high and low-flow condition, a total of 163 and 162 species of phytoplankton, and a total of 62 and 36 species of zooplankton, respectively were observed. The dominant species of phytoplankton communities in low-flow condition metamorphosed from *Cyclotella* sp. to *Navicula* sp. and *Cocconeis placentula*; and *Pediastrum simplex* to *Ankistrodesmus spiralis*. The metamorphosis in zooplankton community was from *Diffugia globulosa* to *Strobilidium* sp. Comparing with other karst water in different climate regimes, the number of plankton species in the subtropical karst wetland was the highest, which is related to the high and stable water temperature, large karst underground spaces, and frequent interaction between groundwater and surface water. The abundance, biomass and community semblance of plankton were increased with an increase in nutrients, precipitating improvement in hydrodynamics and light intensity. This study also suggested that the plankton in karst cave wetlands in subtropical area were characterized by low diversity, attributable to the calcium-rich and weakly alkaline, but poor nutrient status of the water environment, as well as drew attention to the possible functions of wetlands in relation to the quality of connected river.

KEYWORDS

phytoplankton, zooplankton, hydrological conditions, nutrients, karst cave wetlands

Introduction

Wetlands are vital for human survival (Xu et al., 2020), providing most of the global ecosystem services value (Costanza et al., 2014). Wetlands are considered as the “kidney of the Earth” and are very important landscape resource and ecosystem owing to their unique role in regulating the global biogeochemical cycle. About 91% of the total research on wetlands have been devoted to the driving factors of wetland change (Birch et al., 2022), ecosystem service function evaluation (Badamfirooz et al., 2021), wetland protection policy and management (Myers et al., 2013). In these studies, 29% were river wetlands, 10.8% were lake wetlands and 19.5% were compound wetlands (Xu et al., 2020). These wetlands are basically surface wetlands.

Wetlands in karst areas often have huge underground space (karst conduit/cave) or are closely associated groundwater. This kind of “half-ground and half-underground” and “half-light and half-dark” wetland is significantly different from riverine and lacustrine wetlands in terms of hydrological process, biodiversity and biogeochemical cycle (Pipan and Culver 2019). Karst wetland is rarely addressed because the complex hydrological processes are often the focus of karst research. The hydrochemistry of karst wetlands, characterized by rich calcium and bicarbonate, was usually well-known (Ford and Williams, 2007), while the biometrics were rarely involved. Durán et al. (2010) reported the hydrogeological characterisation of a karst wetland and offered an explanatory model of its origin and karstic functioning, as well as pointing to the possible causes of its historical disappearance. 17 wetlands in Pennsylvania, United States were examined the ground-water hydrology to determine the contributing area of wetland source waters (Michael et al., 2003). The studies of plankton in karst wetlands were reported mostly in Mediterranean (Jasprica et al., 2006), temperate forest (Okhapkin et al., 2022), and tropical area (Sánchez et al., 2002).

The national standard for “wetland classification” of the People’s Republic of China defined a unique wetland type as “karst cave wetland”, which means that certain water patches are recharged by groundwater where karst caves or conduits are developed (GB/T 24708-2009). According to the definition, the karst cave wetland may include karst springs, karst pools, open stream sections of underground rivers, streams, and so on. This kind of wetland is sporadic, discontinuous, variable (Lan et al., 2018), and highly dependent on hydrogeology and hydrological conditions. Research on karst cave wetland remains very limited except for several big karst wetlands in South China. A karst spring group in Guangxi has been continuously monitored for 12 years and the changes of plankton community was compared (Wei et al., 2022). The landscape of karst wetland (Huang et al., 2016), heavy metal pollution and health risk assessment (Ba et al., 2022), and the diversity of soil fauna communities (Lan et al., 2018) were the common subject of the existing case studies.

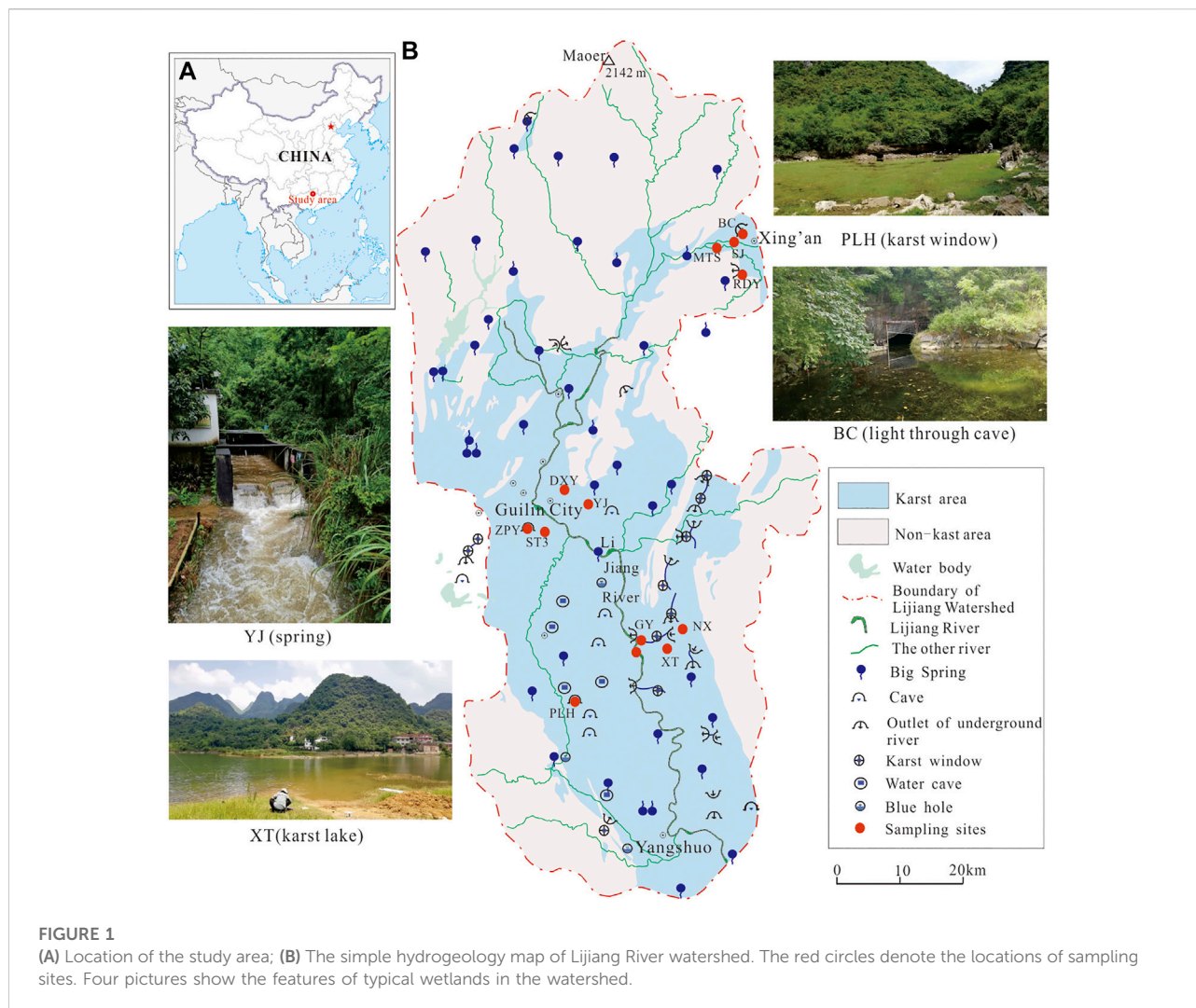
The inorganic chemical evaluation results of karst cave wetlands in the Lijiang River Basin of previous studies have been shown to be basically good (Zhang et al., 2018), but the visible water environment is nonetheless bad in some areas, notably characterized by lack of submerged plants and algae floating debris. In this case, biological factors should be considered to accurately evaluate the water environment of karst wetlands (Guo et al., 2019). Phytoplankton communities give more information on changes in water quality than mere nutrient concentrations or chlorophyll a (Chl-a) concentration. As a component of aquatic ecosystems, phytoplankton has been recognized as an indicator of trophic status and water quality of lakes (Reynolds et al., 2002; Palmer et al., 2015). Phytoplankton succession in open lakes depends on the availability of nutrients, hydraulic retention time, temperature, light intensity, and transparency (Vaulot et al., 2008). Phytoplankton communities are sensitive to changes in their environment, consequently, total biomass and many phytoplankton species are used as indicators of water quality (Reynolds et al., 2002; Brettum and Andersen 2005; Reynolds 2006). Considering specific biogeochemical processes in karst wetlands, we hypothesized that the plankton communities are unique and are sensitive to the environmental changes.

This paper was aimed at studying the plankton community structure and diversity of selected karst cave wetlands in Lijiang River basin. The plankton distribution patterns and their correlations with hydrological dynamics and hydrochemistry were explored, in order to determine the specific type of plankton that flourishes in the wetlands under the special environment of karst groundwater recharged with karst cave, and to provide data on the possible effect of karst cave wetlands on the Lijiang River.

Methods and materials

Study area

Guilin, a city located in Southern China, is famous for its glorious karst landform. It has become one of the world’s prominent tourist destinations and was listed as a world natural heritage site in 2014. Lijiang River (LJ), the largest river in Guilin, runs through the city from south to north. It is a famous scenic spot and an important drinking water source in Guilin City (Shahab et al., 2020). Being one of the tributaries of the Pearl River, Lijiang River is approximately 164 km in length (Figure 1). The catchment area of the Lijiang River comprises the section from Xing’an County to Yangshuo County, with a total basin area of 5,585 km², 49% of which are carbonate area (Zhao 2018). In addition to Huixian, the largest wetlands in the basin, numerous scattered wetlands composed of many karst springs and pools distribute in the basin, which played a critical role in regulating the water volume of the Lijiang River and purifying the water quality. However, at present, research on karst wetlands in the basin is rarely given due attention.



The strata exposed in the Lijiang River Basin are briefly distributed as follows: the northern part is composed of Caledonian Granite and Paleozoic strata such as Sinian, Cambrian, Ordovician and Silurian; the southern part is composed of Devonian and Carboniferous limestone and clastic rock. The middle and lower reaches of Lijiang River is composed mainly of the limestone stratum of Rongxian formation and Upper Devonian of Guilin formation (Miao, 1998).

The topography of headwater and the upper reach area is hilly mountains with an elevation of more than 1,000 m. The middle and lower reaches of the basin are karst landforms, characterized by peak-cluster depressions, valleys, and peak forest plains, with elevations ranging from 100 to 500 m (a.s.l). Clastic rock mountains are located at the north edge of Lijiang River Basin, where the streams are considered as allogeneic river. Allogeneic river enters the karst area, turns into underground river through sinkhole, and becomes karst

creek along Lijiang River or in the plain area. In addition, there are sinkholes (foot caves) in the dissolution plain, which form pools recharged by groundwater. The water patches with a certain dimension and significant water level variation in karst area were then identified as karst cave wetlands.

The flow rate of Lijiang River varies between 12 m³/s and 8,000 m³/s in different conditions, and the annual average discharge is about 120 m³/s (Chen et al., 2011). The climate of the basin is typically a subtropical monsoon with annual average temperature and rainfall of 19.3°C and 1,930 mm, respectively. Overall, 70% of the rainfall occurs between April and July.

Sample collection and analysis

In May and November 2020, which representing high water condition and low water condition, a total of 13 karst cave wetlands were selected for investigation and sampling. The

TABLE 1 Information on the sampling sites.

Sampling site	Information
BC, SJ, RDY, ZPY, GY, DXY, YJ, PLH	With karst caves of different sizes from 10 to 100 m, forming certain area of water patches inside and outside the caves
XT	Lake in karst depression because of sinkhole clogging
ST3	Pool connected with karst aquifer
NX	Stream originated from clastic rock mountain with cobblestone riverbed. Wide: 5 m; water depth: 0.1 m; velocity: 0.303 m/s
MTS	A river channel with Gravel riverbed and dense submerged plants. Wide: 35 m; Water depth: 1.45 m; velocity: 1.102 m/s
LJ	Main river channel of the basin with gravel riverbed and dense submerged plants. Wide: 30–100 m; water depth: 2–3 m

reasons for choosing these 13 sampling sites were to: cover the edge, middle and most developed karst area in the basin including the major hydrogeology types; examine the different human activities and utilization statuses; and consider the report of previous research (Guo et al., 2021). NX, MTS, LJ are stream/ rivers, and the other 10 sites are groundwater with typical karst features (Table 1; Figure 1).

Physicochemical variables, including pH, water temperature (WT), electrical conductivity (EC), pH, oxidation reduction potential (ORP), and dissolved oxygen (DO) were measured employing a Manta multi-parameter water quality instrument (Easy Probe), simultaneously with plankton sampling. The precisions of them are ± 0.10 units, $\pm 0.10^\circ\text{C}$, $\pm 1.0 \mu\text{s}/\text{cm}$, $\pm 20 \text{ mv}$, $\pm 0.1 \text{ mg/L}$, respectively. Ca^{2+} and HCO_3^- were titrated on site using a Calcium Test kit and a Biocarbonate Test kit (Merck KGaA, Germany), with precisions of 2 mg/L and 0.1 mmol/L , respectively. Chl-a in water was measured employing Turner Cyclops-7F, with minimum detection limits of $0.025 \mu \text{ g/L}$.

Water samples (1.5 L) were collected with a Plexiglass water collector (DSC2500, produced by Xiamen Dengxun Co. Ltd.) and stored immediately in cooling boxes before they were transported to the laboratory. The samples were analyzed to determine major ions, including K^+ , Na^+ , Ca^{2+} , Mg^{2+} , Cl^- , SO_4^{2-} , HCO_3^- , total nitrogen (TN), total phosphorus (TP), nitrate (NO_3^-), nitrite (NO_2^-), and ammonia (NH_4^+), according to the standard methods of water analysis monitoring.

5 ml of water sample was collected with sterilized plastic bottle for *Escherichia coli* (*E. coli*) concentration culture. 3M Petrifilm™ *E. coli*/coliform count plates (5 ml) were employed for on-site inoculation, and the plates were placed in an incubator for 24 h at a temperature of 37°C .

A plankton net (10- μm mesh) was applied for several times at a total of 6 min to collect samples at each site from 1 m depth underwater. Thereafter, each 150 ml samples were stored in two plastic bottles and sent to the laboratory for the phytoplankton (fixed with 10% Lugol's solution) and the zooplankton (fixed with 10% formaldehyde solution) identification. Another 1,000 ml of water sample was collected into a bottle, containing 1% formaldehyde solution, and then stored in the laboratory for 48 h for quantitative phytoplankton analyses.

20,000 ml of water was collected and then filtered by the plankton net to collect 100 ml samples for quantitative zooplankton analyses. The identification of phytoplankton and zooplankton was completely followed by the trade standard of Chinese (The trade standard of the People's Republic of China), and was referenced to ensure accurate species determination (Hu et al., 2006). The biomass of phytoplankton and zooplankton were converted by volume, and the specific gravity was taken as 1. Results were expressed as cell density of phytoplankton per liter (cells./L) and individual of zooplankton per liter (ind./L). Each cell of filamentous algae was counted as a single cell.

Statistical analysis was carried out using the Excel and SPSS 11.0 package. Relationships between the plankton species and physical-chemical variables were analyzed using CANOCO 4.5 software (Microcomputer Power, New York, United States). Before analysis, all data (except pH) were transformed by $\lg(x + 1)$ to satisfy the normal distribution.

Hydrological dynamics monitoring

Water level, depth, velocity, and the change of wetland area were surveyed each time water was sampled. Two different kinds of karst wetlands, belonging to spring and foot cave types in the Lijiang River Basin were selected to perform a 1-year hydrological monitoring. The water levels, WT, and EC were automatically recorded employing a Solinst Levellogger (Solinst Canada Ltd.), with precisions of 0.41 cm and 0.1°C , respectively. A Solinst barometer was also used for air pressure correction.

Results

Diversity of wetland type in lijiang river basin

There are many wetland classification methods for conservation and management goals (Finlayson and Valk, 1995). Wetlands can even be divided into flowing/relatively static wetlands or large/small wetland according to the speed of water renewal or the area. A total of 13 wetlands in the Lijiang River basin were classified based on the

TABLE 2 Diversity of the studied wetlands and their hydrological character.

Type	Sub-type	Sampling points	Hydrological character
Peak-cluster type	Light through cave	DXY, BC	Intermittent of light and dark, easily to be polluted
	spring/subterranean river	SJ, RDY, GY, YJ	Strong groundwater dynamics and large hydrological dynamics
	Karst window	PLH	Small area and large depth of water body, and close to drying up in dry season
	Karst lake	XT	Large area and big depth of water body
Peak-forest type	Foot cave	ZPY	Groundwater collecting point in the plain
	Pool	ST3	Recharged by foot cave, small water depth
Compound type	Stream/River	NX, MTS, LJ	Strong water dynamics and large water section

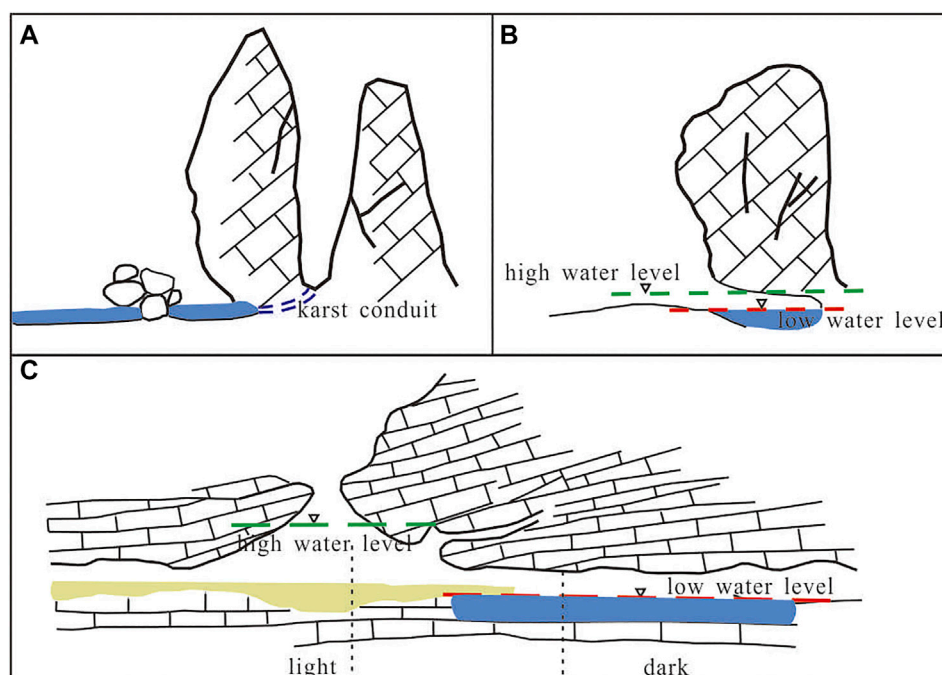


FIGURE 2

The sketch map of three main karst cave wetlands. (A) spring/subterranean river-type wetland; (B) foot-cave-type wetland; (C) karst window-type wetland.

landform and hydrogeology because the character of wetland in karst area is obviously affected by topographic types and hydrogeological conditions, and the distribution of karst wetlands is closely related to the karst development and hydrogeological conditions. Firstly, according to the topographic feature, wetlands could be classified into peak-cluster and peak forest types. River was considered as a combination type. Secondly, based on hydrogeological conditions, the peak-cluster type could further be classified into four types: light through cave, spring, karst window, and karst lake. The peak forest type could further be classified into foot cave and pool. The diversity of wetlands and their hydrological character are listed in Table 2.

The particularity of karst cave wetlands includes locating in karst areas; having a certain scale of karst cave or conduit space; recharging

by karst groundwater or having a close connection with karst groundwater. The schematic diagrams of three main cave wetlands are presented in Figure 2, showing the characteristic of “half-light and half-dark” and “half-surface and half -underground” (Figure 2).

Characteristics of physico-chemical variables and E.coli

The main environmental variables in wetlands are shown in Table 3. The results showed that the wetlands in the study area were weakly alkaline as a whole. Water temperature ranged

TABLE 3 Physico-chemical variables of selected wetlands.

Sites	Mon	T (°C)	pH	ORP (mv)	EC (µs/cm)	DO	Ca ²⁺	HCO ₃ ⁻	TP	NO ₃ ⁻	NH ₄ ⁺	NO ₂ ⁻	Chl-a
BC	May	21.11	7.47	188.5	365.5	5.92	81	157.5	0.07	8.86	0.12	0.03	2.13
	November	18.40	7.35	334.7	463	5.87	92	264.6	0.04	0.16	0.81	2.71	
SJ	May	19.50	7.53	182.4	224.9	9.08	48	126	0.09	4.43	0.24	1.30	0.75
	November	19.75	7.32	294.3	457.7	8.65	100	289.8	0.04	0.12	0.05	3.01	
MTS	May	23.13	7.61	162.5	234.9	7.35	47	113.4	0.07	8.10	0.30	0.04	0.78
	November	19.15	7.51	291.1	308.9	8.1	60	163.8	0.03	0.12	0.83	7.13	
RDY	May	19.92	7.63	186.5	158.5	8.7	30	81.9	0.06	7.13	0.06	0.01	0.06
	November	18.55	7.66	275	241.7	8.79	44	138.6	0.02	0.11	0.02	7.71	
ZPY	May	20.82	6.91	185.7	560.2	2.47	105	258.3	0.02	9.52	0.05		0.06
	November	21.59	6.83	320	582.8	5.01			0.02	0.09	0.02	1.44	
ST3	May	28.21	7.36	207.5	434.3	8.31	72	214.2	0.05	4.05	0.25	0.11	2.38
	November	21.43	8.03	295.8	333.6	9.13	54	94.5	0.03	0.23	0.04	0.00	
NX	May	21.09	7.76	176.4	73.8	8.55	10	37.8	0.02	4.28	0.03		0.28
	November	20.01	7.76	298.9	100.2	8.63	18	50.4	0.03	0.09	0.01	2.91	
XT	May	27.36	8.07	186.5	250.6	8.56	52	144.9	0.03	5.49	0.31	0.04	4.01
	November	20.76	7.54	325.4	299	6.91	62	189	0.03	1.27	0.11	0.49	
GY	May	20.44	7.99	189.9	306	8.75	66	189	0.02	7.48	0.19	0.02	0.16
	November	19.99	7.98	316.6	290.6	8.63	62	189	0.04	0.15	0.03	6.91	
DXY	May	24.85	7.12	165	432	0.86	82	239.4	0.19	4.56	2.10	1.67	23.4
	November	18.93	7.21	345.7	557.8	4.47							
YJ	May	19.35	7.16	243.5	386.6	8.3	85	239.4	0.02	4.20	0.12		0.18
	November	19.54	6.86	380.1	497.5	8.14	114	352.8	0.03	0.09	0.02	1.21	
PLH	May	22.70	7.37	299	332.4	5.26	73	195.3	0.07	8.28	0.18	0.23	0.18
	November	21.14	7.24	311.4	370.5	5.65	82	245.7	0.03	0.08	0.01	3.53	
LJ	May	24.05	7.42	190.5	189.3	7.36	32	88.2	0.07	7.84	0.12		0.82
	November	20.24	8.53	298.7	138	11.36	24	63	0.03	0.12	0.12	4.28	

from 18.55 to 28.21°C. The EC of wetlands ranged from 224.9 to 582.8 µs/cm, and the lowest value of EC of river was only 73.8 µs/cm in allogeneic stream. All the ORP had positive values. The DO content was between 0.86 and 11.36 mg/L. The lowest DO occurred at DXY. Ca²⁺ and HCO₃⁻ were the main ions. The Ca²⁺ content of rivers ranged from 10 to 60 mg/L, while the Ca²⁺ contents of wetlands were between 30 and 114 mg/L. The HCO₃⁻ content of rivers were between 50.4 and 163.8 mg/L, and the HCO₃⁻ content of other karst groundwater ranged from 94.5 to 352.8 mg/L. The values of turbidity ranged from 1.2 to 37.3 NTU, with the highest value recorded in DXY. The content of TP was basically low, with values of 0.02–0.19 mg/L. The NO₃⁻ content was between 0.09 and 9.52 mg/L and the content of NH₄⁺ was 0.01–2.10 mg/L, with the highest value recorded in DXY. NO₂⁻ could not be detected in five samples, and the rest samples had a high value of 7.71 mg/L. Significant difference in Chl-a content among sites ranged from 0.06 to 23.4 µg/L, with the highest content recorded in DXY.

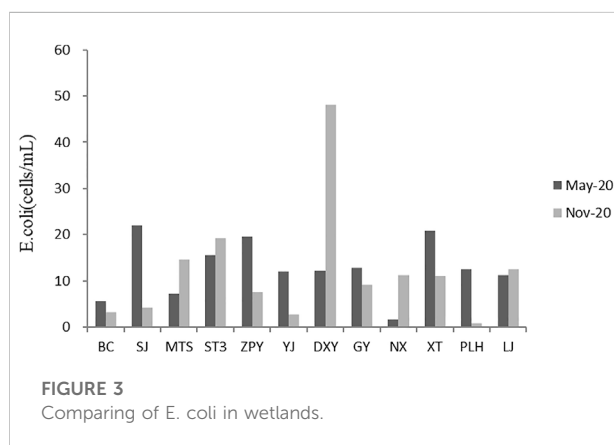


FIGURE 3 Comparing of E. coli in wetlands.

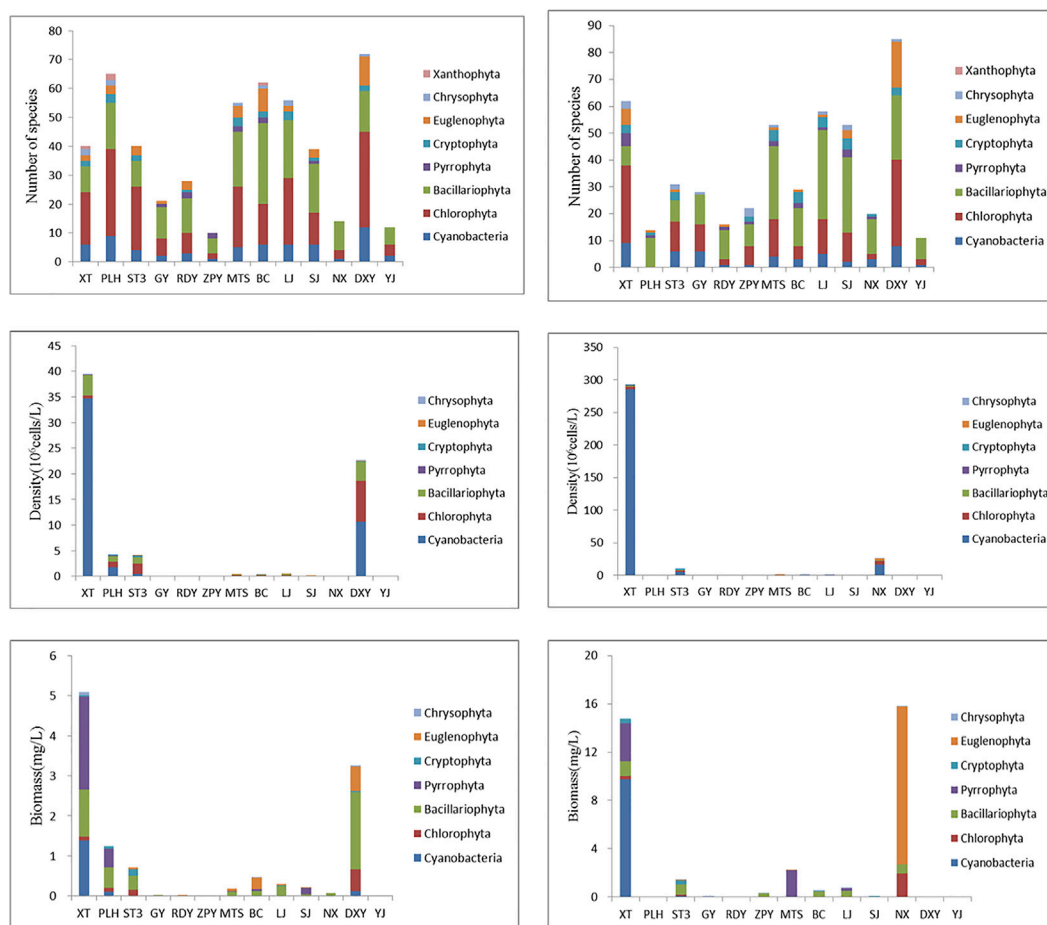


FIGURE 4 Phytoplankton community composition of each sampling sites. The left column was the results in May and the right column was results in November.

DXY had the highest average values of *E. coli* concentration with 50 cells/mL. The sampling sites were polluted to varying degree according to the concentration of *E. coli* except for YJ and NX.

There was little difference in water chemical parameters among the samples. However, evaluation of biotic indicators such as *E. coli* and Chl-a indicated significant difference among samples. Sampling points of DXY, XT, SJ, ZPY, and ST3 were in relatively bad water environment. All the sampling points lacked submerged plants; with the *E. coli* in excess of 10 cells/mL.

Phytoplankton

In May, a total of 163 species of phytoplankton were identified, including 20 species of *Cyanobacteria*, 67 species of *Chlorophyta*, 43 species of *Bacillariophyta*, six species of *Pyrrophyta*, four species of

Cryptophyta, 15 species of *Euglenophyta*, four species of *Chrysophyta*, four species of *Xanthophyta*. The density of *Cyanobacteria* was 48.07×10^6 cells/L, *Chlorophyta* was 11.97×10^6 cells/L, *Bacillariophyta* was 10.54×10^6 cells/L. The total density of the rest species was 1.49×10^6 cells/L. The order of biomass in each phylum was: *Bacillariophyta* (4.58 mg/L) > *Pyrrophyta* (2.99 mg/L) > *Cyanobacteria* (1.63 mg/L) > *Euglenophyta* (1.07 mg/L) > *Chlorophyta* (0.92 mg/L) > *Chrysophyta* (0.11 mg/L).

In November, a total of 162 species of phytoplankton were identified, including 14 species of *Cyanobacteria*, 56 species of *Chlorophyta*, 55 species of *Bacillariophyta*, six species of *Pyrrophyta*, five species of *Cryptophyta*, 21 species of *Euglenophyta*, five species of *Chrysophyta*. The cells density of *Cyanobacteria* was 308.10×10^6 cells/L, and *Chlorophyta* was 11.23×10^6 cells/L, *Bacillariophyta* was 5.32×10^6 cells/L. The total of the rest species was 5.65×10^6 cells/L. The order of biomass in each phylum was: *Euglenophyta* (13.11 mg/L) > *Cyanobacteria* (9.89 mg/L) > *Pyrrophyta* (5.62 mg/L) >

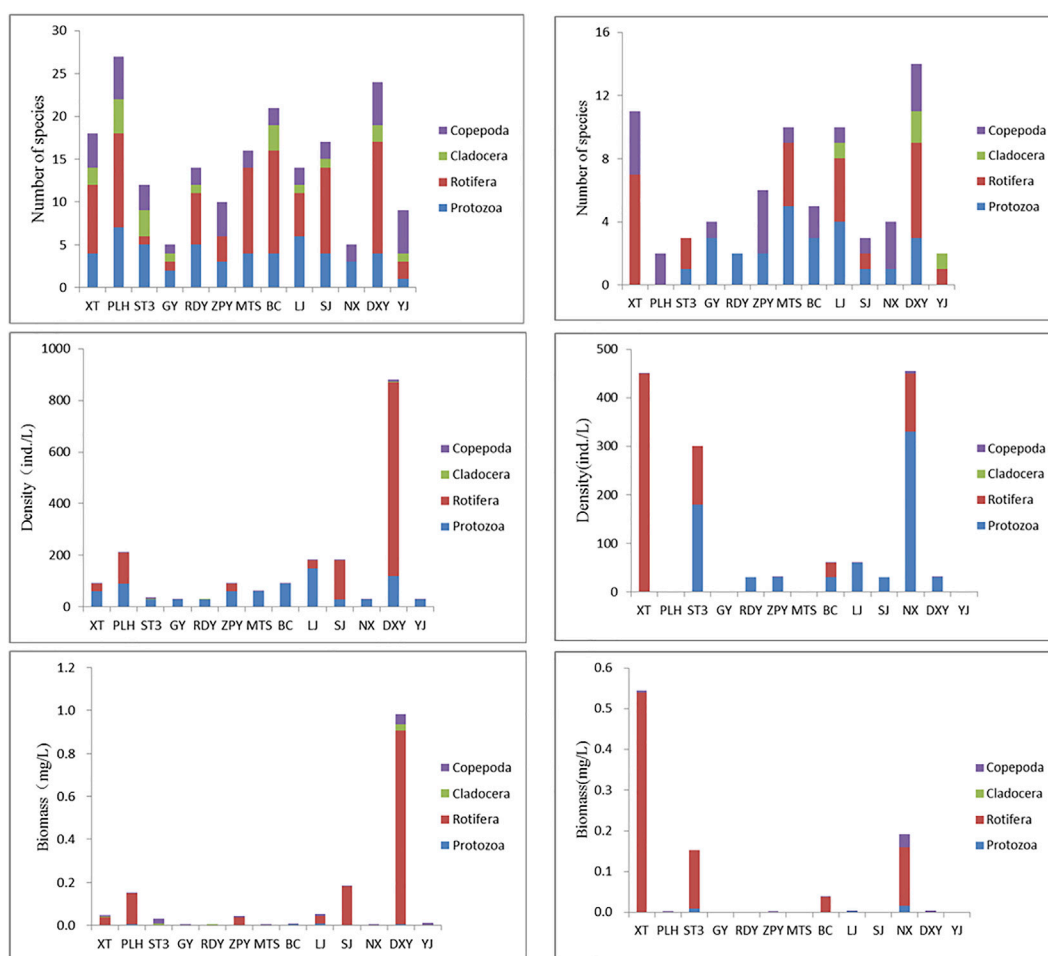


FIGURE 5
Zooplankton community composition of each sampling sites. The left column was the results in May and the right column was results in November.

Bacillariophyta (4.09 mg/L) > Chlorophyta (2.40 mg/L) > Cryptophyta (0.84 mg/L) > Chrysophyta (0.06 mg/L).

The number of phytoplankton species was always high in DXY and XT, and low in YJ, NX, and ZPY in either May or November (Figure 4). Comparatively, the density and biomass of phytoplankton were highest at XT and DXY in May and highest at XT and NX in November.

Zooplankton

In May, a total of 62 zooplankton species were observed in all samples, which consisted of 15 species protozoon, 33 species rotifers, nine species cladocerans, five species copepods. Totally 36 species of zooplankton were observed in November, which consisted of 10 species protozoon, 20 species rotifers, four species cladocerans, two species copepods.

In May, the total of zooplankton density was 1941.43 ind./L, and the order of each group was: rotifers (1,110.00 ind./L) > protozoon (810 ind./L) > copepods (19.50 ind./L) > cladocerans (1.93 ind./L). The biomass of zooplankton was 1.52 mg/L, and the order of each group was: rotifers 1.33 mg/L > copepods 0.11 mg/L > protozoon 0.04 mg/L > cladocerans 0.04 mg/L. In November, the total of zooplankton density was 1,446.60 ind./L, and the order of each group was: protozoon (720 ind./L) > rotifers (720 ind./L) > cladocerans (0.05 ind./L) > copepods (6.55 ind./L). The biomass of zooplankton was 0.95 mg/L, and the order of each group was: protozoon (0.04 mg/L) > rotifers (0.86 mg/L) > copepods (0.04 mg/L) > cladocerans (0.0 mg/L).

In May, the number of zooplankton species was the highest at PLH, followed by DXY and BC, while DXY had the highest species in November, followed by XT. The zooplankton density and biomass showed highest values at DXY in May, while XT and NX had the highest values in November (Figure 5).

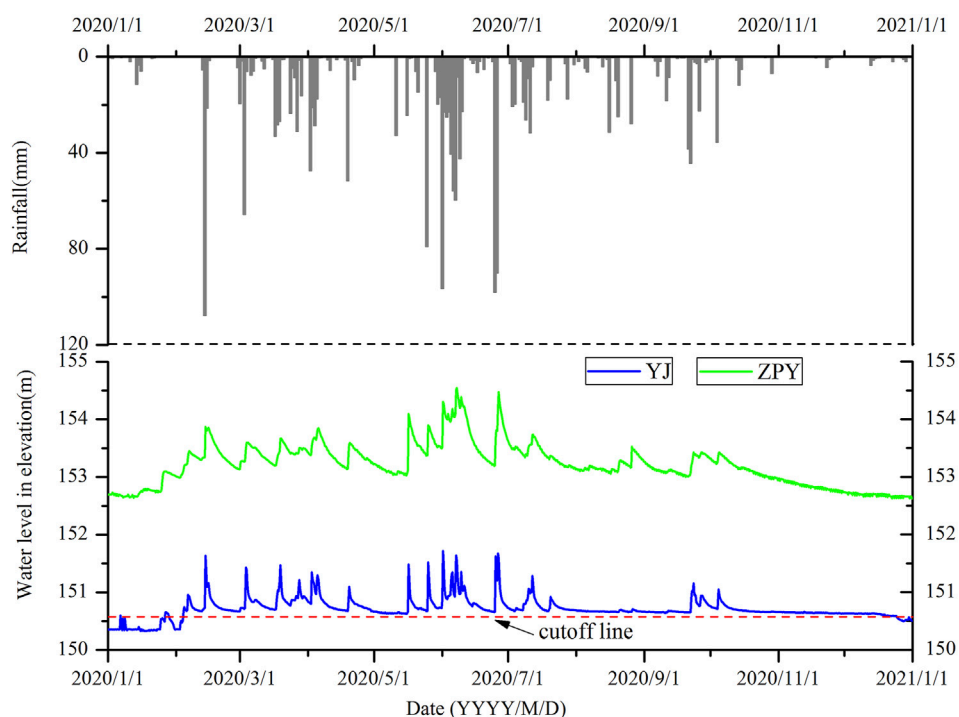


FIGURE 6
The hydrological process of two typical wetlands in Lijiang River watershed.

Discussion

The influence of hydrogeology background and hydrological condition on plankton

Previous studies have shown that hydrological connectivity is one of the factors determining the plankton community structure (Xiao et al., 2021). The hydrological conditions were distinctly different in different wetlands' type. For the wetland in peak-cluster depression, groundwater recharges more quickly than those in peak-forest plain. For instance, the dye tracer tests showed that the groundwater velocity was 10^2 – 10^4 m/d in YJ spring, while groundwater movement velocity in ZPY, which belongs to peak-forest plain in topographic state, was 10 – 10^2 m/d (Jiang et al., 2016). According to the long-term monitoring outcomes of selected groundwater in Lijiang River Basin, the annual hydrodynamics of groundwater level in spring in peak-cluster depression was between 1 and 2 m, and 1–3 m in foot cave type wetland in peak-forest plain. The variation of water level in karst window was more significant. For example, at the PLH wetland, the water level in rainy season was 7 m higher than that in dry season.

Both YJ spring and ZPY foot cave are located in the Lijiang River Basin, with a distance of only 12 km. However, YJ belongs

to the karst spring system of peak-cluster depression, while ZPY belongs to the foot cave system of peak-forest plain. The response of groundwater level dynamics to rainfall was basically consistent, indicating that rainwater was the primary recharge source of groundwater in this area. The difference was due to the fact that the hydrological curve of YJ spring was steeper and sharper, and the attenuation of the groundwater level peak of ZPY was slower (Figure 6), indicating that the karst spring system in peak-cluster depression responds more drastically to rainfall and has the characteristics of rising and decline quickly, while the regulation and storage capacity of the foot cave type in peak-forest plain is stronger.

Another characteristic of karst cave wetland is that the hydrologic dynamics changes greatly. The flow difference between rainy season and dry season is significant, and the flow speed of groundwater is also different. In rainy season, the water level of wetland is high, and the flow is fast. In dry season, the water level is shallow or even dry. For instance, the discharge rate of YJ spring was 12.8 L/s on May 20 and 0.4 L/s on 13 November 2020. The water condition in May and November was considered as high-flow and low-flow, respectively due to the average discharge rate which was 1–2 L/s during the normal water flow period. The change in water level at individual sampling points led to appropriate adjustment in sampling locations. For example, groundwater only accumulated at the

cave entrance of the YJ spring in November. Consequently, the sampling in November was done at the cave entrance, while the sampling in May was done at the flowing stream away from the entrance of 20 m. This factor could probably affect the community composition and biomass of plankton, culminating in the metamorphosis of dominant species in high water level period and low water level period. The predominant species were *Achnanthes*, *Nitzschia*, and *Gomphonema* species of Bacillariophyta in the two periods (Supplementary Table S1). The difference in Bacillariophyta metamorphosed from *Cyclotella* sp. to *Navicula* sp. and *Cocconeis placentula* between May and November. The dominant species of Chlorophyta metamorphosed from *Pediastrum simplex* to *Ankistrodesmus spiralis*. More importantly, *Pseudoanabaena* species of cyanobacteria showed up in November. *Pseudoanabaena* is the dominant species of cyanobacteria bloom phenomenon. It thrives in low water level and slow flowing water conditions, indicating that hydrological condition is a shaping force in structuring the plankton community in the water systems (Phlips et al., 2008).

Relationships between community composition and hydrochemistry environmental variables in karst cave wetlands

Detrended correspondence analysis (DCA) was intended to find out the relationship between environmental variables and plankton distribution. The DCA analysis results showed the eigenvalue of axis one and axis two were 0.779 and 0.392 for phytoplankton, respectively. And the first two axes contributed 25.2% of variance of phytoplankton species data and 37.9% of species-environment relation. For zooplankton, the eigenvalue of the axis one and axis two were 0.914 and 0.652, respectively. And the first two axes contributed 21.3% of variance of phytoplankton species data and 36.6% of species-environment relation.

DCA of plankton abundance and environmental factors showed that water temperature, ORP, and NO_3^- were the main factors influencing the abundance of phytoplankton, followed by NH_4^+ , NO_2^- , DO, and turbidity (Figure 7). Most of the phytoplankton species were controlled by water temperature, and only a few species were controlled by ORP, NO_3^- , and turbidity. Water temperature, pH, turbidity and NO_2^- were the main factors that affected the abundance of zooplankton, followed by NO_3^- and COD. Most of the zooplankton species were closed related to water temperature, turbidity and NO_2^- , and only a few species were controlled by water temperature and pH. Furthermore, there were some other species which did not correlate with these environmental variables.

For wetlands recharged by karst groundwater, there was little difference between the water temperature inside the aquifer and

the groundwater outlet. The temperature was relatively stable, mostly distributed between 17 and 19°C in the study area. In theory, therefore, water temperature should not be a restriction factor for the plankton species, as has been showed in other wetlands (Sun and Wang, 2021). However, the measured water temperature ranged from 18.55 to 28.21°C, which was due to the transformation of water bodies/pools of different sizes after groundwater outflow. The rising range of water temperature was different due to the effect of many factors such as light, depth of water patches and so on (Jin et al., 2022). We did not measure the exact outlet of groundwater frequently due to limitation of site conditions. Consequently, the water temperature was higher than that of the aquifer, which explains why the water temperature was also a factor controlling phytoplankton and zooplankton species.

To understand the level of plankton species or abundance in the subtropical karst wetland, numbers of plankton species in karst water under several climate types in the world were compared. It was found that the number of plankton species in the subtropical karst wetland in southern China was the highest (Table 4). This may be related to the high and stable water temperature of the subtropical karst wetland, the large karst underground spaces (dominated by karst conduits and caves), and the frequent interaction between groundwater and surface water. The species numbers or dominant species of phytoplankton have a great relationship with the water environment and nutrient elements (Phlips et al., 2008). In clean water, Bacillariophyta usually accounts for an absolute predominance (Góngora and Silveira, 2006). Although the phyla with the largest proportion of zooplankton are rotifers, the dominant species are different, which are related to the biomass of phytoplankton, groundwater flow, and other environmental conditions.

Based on the relationship between sample distribution and phytoplankton species, NX and SJ were grouped as one type. BC and RDY were grouped into two separate types, and the rest could be grouped into one type. For zooplankton, the relationship between sample distribution and zooplankton species were similar in LJ, MTS, XT, which could be grouped into one type. ST3, BC, NX, GY were grouped into another type and the rest sampling were grouped into other types. From the classification results, it was found that environmental factors did affect the community structure of plankton. Among the factors, plankton had the greatest relationship with turbidity, NH_4^+ , NO_2^- , and these indicators reflect the impact of local land use patterns and human activities on the wetland water environment. The chemical fertilizer employed in farmland farming and domestic sewage discharged were the main reasons for the deterioration in water chemical parameters. They were also the primary factors for determining whether there were submerged plants and plankton communities in the wetland. The shifting of dominant species was also associated with the water environment. The predominant species of zooplankton

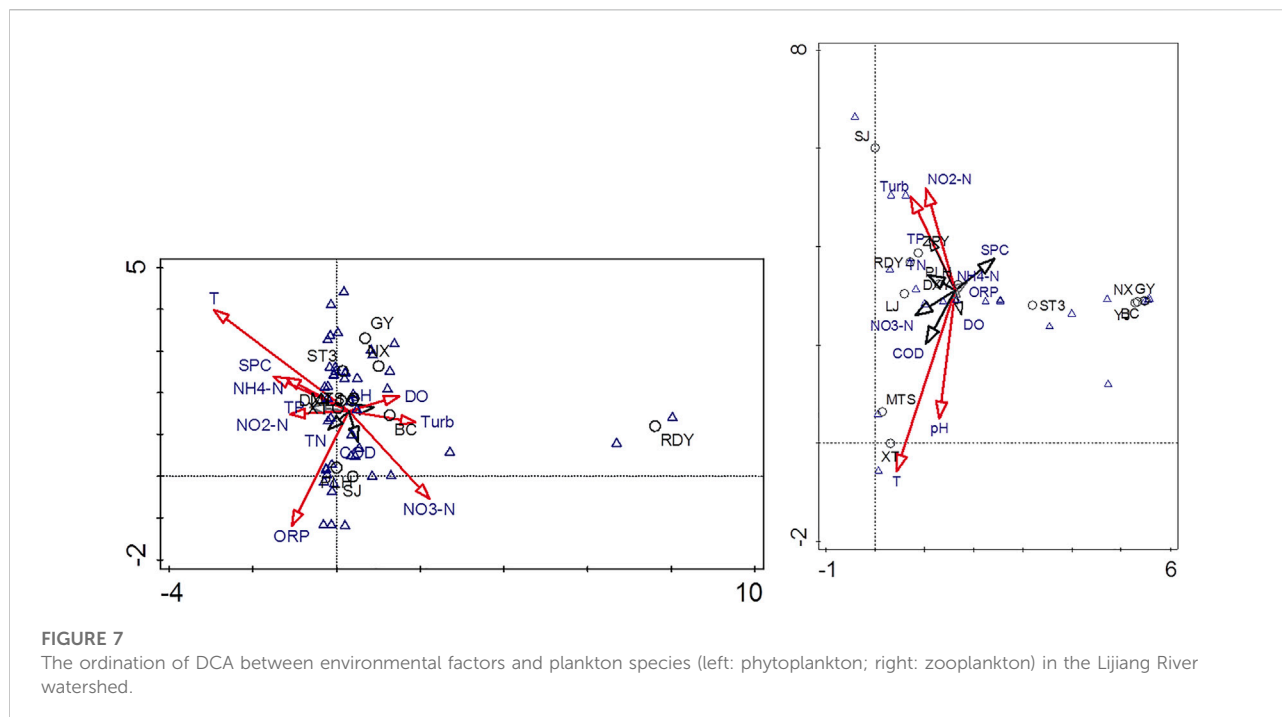


TABLE 4 Comparison of plankton species in karst water under several climate conditions.

Plankton	Country	Climate	Habitat	Number of species	Maximum phylum
Phytoplankton	Croatia ⁽¹⁾	Mediterranean	Karst lake	86	<i>Cyanobacteria</i>
	Russia ⁽²⁾	Temperate forest	Karst lake	133	<i>Bacillariophyta</i>
	Turkey ⁽³⁾	Mediterranean	Karst spring	49	<i>Bacillariophyta</i>
	Mexico ⁽⁴⁾	Tropical	Cave	78	<i>Xanthophyta</i>
Zooplankton	Greece ⁽⁵⁾	Mediterranean	Karst lake	33	Rotifer
	Turkey ⁽³⁾	Mediterranean	Karst spring	24	Rotifer

Note: Square brackets represent references. [1], Jasprica et al., 2006; [2], Okhapkin et al., 2022; [3], Demir and Kirkagac, 2005; [4], Sánchez et al., 2002; [5], Chalkia et al., 2012.

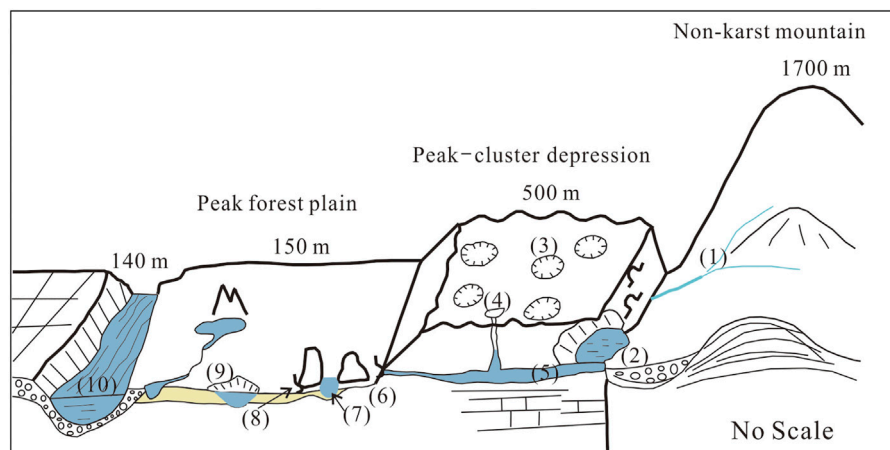
were *nauplius* and copepod larva of cyclops of copepods. The difference was that, in May, the secondary dominant species were *Arcella vulgaris* and *Diffugia globulosa*, and some rotifers species. In November, the secondary dominant species were *Strobilidium sp.* and *Arcella megastoma* of protozoon (Supplementary Table S2). The water environment factor is one of the factors leading to the change in plankton community structure since the oligotrophic water patch is the most suitable living environment for *Arcella vulgaris*.

The indicative significance of plankton in karst cave wetlands to the water environment of river

Non-karst area was distributed in the edge of the Lijiang River Basin, and the karst area was distributed in the middle and

the southern region of the basin. Rivers from the non-karst area entered the karst area, resulting in high karst development in the middle and southern regions of the basin, forming two typical karst landscapes: peak-cluster depression and peak-forest plain. The karst formations in peak-cluster depression were sinkhole, karst spring/subterranean river, karst lake, and karst window; while karst formation in peak-forest plain were dominated by light through cave and foot cave (Figure 8). All these karst formations, along with karst conduits, were the main water storage space. The investigation showed that numerous karst cave wetlands were distributed in the Lijiang River Basin because of the well-developed karst.

For the 13 sampling sites, LJ and one of its primary tributaries- MTS, and NX belonged to the river type of wetlands. The other 10 sampling sites were attributed to small and dispersed wetlands. NX river originates from the mountain stream with an altitude of 1701 m, flows through 10 km to the



(1) Allogenic stream; (2) Karst lake; (3) Doline; (4) Karst window; (5) Karst conduit; (6) Spring/ underground river; (7) Light through Cave; (8) Foot cave; (9) Pool; (10) River

FIGURE 8
The sketch map of karst wetlands and their relationship between rivers in Lijiang River watershed.

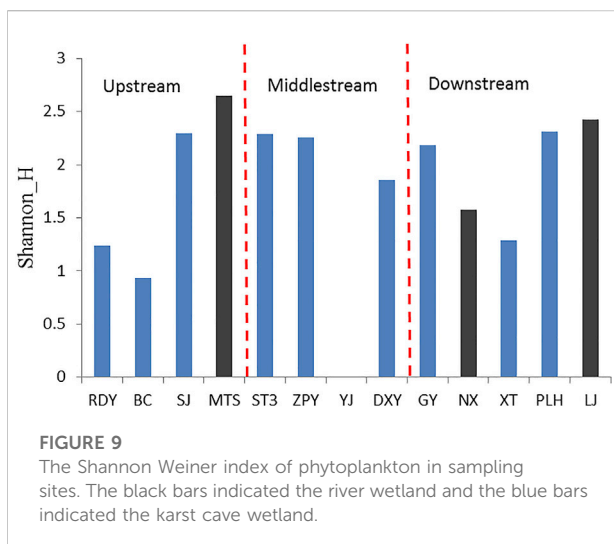


FIGURE 9
The Shannon Weiner index of phytoplankton in sampling sites. The black bars indicated the river wetland and the blue bars indicated the karst cave wetland.

sampling place, and then goes underground after about 2 km. One part became the source of XT Lake and the other part is the source of GY subterranean river. A total of 15 and 20 species of phytoplankton were identified in NX in May and November 2020, respectively. By way of comparison, the richness and diversity of plankton in MST were almost equivalent to those of LJ River, with higher average species of plankton compared to other wetlands. From the viewpoint of spaces, the diversity of phytoplankton was higher in the downstream of the basin than those in the edge and the middle of the river basin (Figure 9).

From the hydrological relationship between wetland and LJ in the watershed scale, NX is a stream from the clastic rock mountain, and MTS is the primary tributary of LJ River in the

plain. NX had the least number of plankton species, which may be attributed to the fact that the source of the stream is the mountainous area covered with forest, the nutrient in the stream is poor, human activities are slight, and the riverbed is cobblestone, with strong hydrodynamic force and no submerged plants. In this case, plankton abundance was low and water quality was excellent. MTS is a secondary river in the plain area. The land use type included a large area of farmland, orchards and villages. The river experienced a gradual increase in nutrients. However, due to strong hydrodynamic force, the river was also rich in submerged plants, and plankton could not bloom in large numbers. LJ is the largest river in the Guilin basin. As a world-famous scenic spot, LJ has been well protected. However, as the river flows through urban and rural areas, it inevitably receives the input of various nutrients. Lijiang River has a wide section, with large flow in rainy season and plenty of submerged plants. Consequently, it still has strong self-purification ability. The species of plankton remain at a medium level and the species diversity is high, implying a relatively healthy river.

Although the connections among these wetlands were low from the viewpoint of spaces, the water environment of wetlands would have effects on the whole aquifer or the basin on the long-run, because of the frequent groundwater—surface water interaction in the karst water system. Basically, the present condition of the water environment in karst wetlands is good, but the deterioration of the water environment caused by local pollution still exist, resulting in a large number of plankton species but low diversity, such as DXY and BC. Therefore, the impact on the aquatic ecological environment of the Lijiang River Basin needs to be evaluated through continuous monitoring of the plankton.

Conclusion

This study was carried out in diverse types of karst wetlands in Lijiang River Basin, a well-known karst scenic landscape in the world. We analyzed the relationship between plankton distribution patterns and hydrological conditions and discussed the effect of hydrochemistry variances on the plankton community structures. Abundance and biomass of phytoplankton and zooplankton were highly related to the nutrients and hydrodynamics of wetlands: highest quantities were detected at the light type through caves which showed high nutrients and weak hydrodynamics conditions; lowest amounts were detected at spring type original from karst mountain where human activities are low. *Achnanthes* sp., *Cyclotella* sp., *Cocconeis placentula*, *Gomphonema* sp. dominated the phytoplankton community, and copepod larva of cyclops and nauplius dominated the zooplankton community. High water flow favoured *Cyclotella* sp. and *Pediastrum*, while low water flow favoured *Navicula* and *Ankistrodesmus spiralis*. *Arcella vulgaris* and *Diffflugia globulosa* were favored by high water flow, while *Strobilidium* sp. and *Arcella megastoma* bloomed in low water flow conditions.

This study suggests that the plankton in karst cave wetlands of subtropical area is characterized by relatively high number of species, comparing to other karst water in different climate regimes. However, it has low diversity of plankton, which can be attributed to the water environment that is rich in calcium, alkali but poor in nutrients. Moreover, the “half-surface and half-underground” wetlands, combined with limited light and low dissolved oxygen was not conducive for the growth of macrophyte, but might promote the growth of plankton. Therefore, the plankton distribution patterns in karst cave wetlands help understand the vulnerability of karst ecosystem. In addition, it is necessary to safeguard the native submerged plants to inhibit the growth of cyanobacterial phytoplankton such as *pseudoanabaena*.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

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Author contributions

FG: Investigation, Data curation, Writing- original draft preparation, Editing. GJ: Conceptualization, Investigation, Writing-reviewing, Revision. FL: Sampling, Data curation.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fenvs.2022.970485/full#supplementary-material>

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