



Research papers

Primary productivity and seasonal dynamics of planktonic algae species composition in karst surface waters under different land uses

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ABSTRACT

Human activities have altered land use patterns and, thus, the hydrochemical features (e.g., $\text{CO}_{2(\text{aq})}$, NO_3^- , and PO_4^{3-}) of watersheds, influencing both the C cycle and the quality of water (e.g., eutrophication) within them. The ways in which primary productivity and the dynamics of planktonic algae species composition are related to these hydrochemical changes remains unclear, especially in karst waters with high pH and high concentrations of Ca^{2+} and dissolved inorganic C. In this study, we used the Shawan Karst Test Site, Puding, SW China to analyze the influences of the NO_3^- , PO_4^{3-} , and $\text{CO}_{2(\text{aq})}$ concentrations on the primary productivity and dynamics of planktonic algae species composition in five spring-fed ponds under different land use patterns at the site during 2018. Our results show the following. (1) Agricultural farming increased the input of NO_3^- , PO_4^{3-} , and $\text{CO}_{2(\text{aq})}$ from springs to the ponds (i.e., surface waters); meanwhile, shrub and grass lands reduced the input of NO_3^- and PO_4^{3-} , but significantly increased the input of $\text{CO}_{2(\text{aq})}$ to the water. (2) The total number of planktonic algal cells was mainly related to $\text{CO}_{2(\text{aq})}$ in January (i.e., the dormant period), but mainly related to NO_3^- and PO_4^{3-} in July (i.e., the growth period); this possibly due to the seasonal succession of phytoplanktonic algae species sensitivity of different nutrient elements. (3) In the dormant period, the system is dominated by the phytoplanktonic algal cells sensitive to $\text{CO}_{2(\text{aq})}$, especially in grass and shrub ponds. In the growth period, the system is dominated by the planktonic algal cells sensitive to NO_3^- and PO_4^{3-} ; however, they are far less than others in grass and shrub ponds. Thus, we predict that groundwaters with relatively high $\text{CO}_{2(\text{aq})}$ and low NO_3^- and PO_4^{3-} concentrations (resulting from natural afforestation in karst areas) may help alleviate eutrophication and increase C sequestration in the corresponding surface waters, indicating the importance of land-use adjustment in the management of water quality and carbon cycle.

1. Introduction

Land use patterns are among the main factors influencing ecosystem functions, since they can alter the biogeochemical cycles of elements in the system (Vitousek et al., 1997; DeFries et al., 2004). Due to the rapid growth of the global population, the demand for new lands for human use has gradually increased. In fact, a large number of non-agricultural lands have been converted into agricultural lands, causing the discharge of large amounts of N and P (i.e., nutrient sources for phytoplankton) into surface waters (Hayes et al., 2015; MacDonald et al., 2012). However, the conversion of farmlands to plant secondary forests can reduce the output of N and P at the watershed scale (Hedin et al.,

1995). Notably, changes in land use patterns in karst areas can affect the concentrations of dissolved inorganic C (DIC, including $\text{CO}_{2(\text{aq})}$) in spring and pond surface waters, (Chen et al., 2017; Zeng et al., 2017). Under the coupling interaction of water, vegetation, soil, and microbes, the dissolution of bedrock in karst areas causes an increase in the input of DIC to surface water (Zhao et al., 2010). This can be assimilated as nutrients, promoting the growth and possibly causing outbreaks of planktonic algae (Visser et al., 2016; Chen et al., 2017; Zeng et al., 2019).

Clearly, changes in nutrient input affect the primary productivity and structure of planktonic algae over spatial and temporal scales (Jiang et al., 2014; Hayes et al., 2015), which, in turn, are linked to the

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structure and function of aquatic ecosystems (Smith et al., 1999; DeFries et al., 2004). The influence of C, N, and P limitation in aquatic ecosystems has been debated for decades. Studies have shown that C, N, and P can all act as main limiting factors for phytoplankton growth under different environmental conditions (Schindler, 1977; Smith, 1983; Schindler et al., 2016; Visser et al., 2016). Previous studies have shown that *Bacillariophyta* grows better than *Cyanophyta* and *Chlorophyta* under relatively low temperatures (T) (Paerl and Huisman, 2008), while *Cyanophyta* and *Chlorophyta* are more likely to outbreak in summer (under relatively high temperature) (Guo et al., 2014) and in nutrient-rich waters (Beman et al., 2005). In addition, cyanobacteria species tend to dominate phytoplankton assemblages in low-N waters, thanks to their ability of fixing N (Smith, 1983). The presence of N-fixing planktonic algae can partially alleviate the N limitation; meanwhile, under increasing P consumption, P will become the main limiting factor for the aquatic system productivity (Schindler, 1977). Changes in the storage pattern of P in soil can further contribute to the occurrence of P limitation (Walker and Syers, 1976). C can also be an important limiting factor for planktonic algae growth (Shi et al., 2017); however, some algae can use C concentrating mechanisms (CCM) to gain a competitive advantage (Reinfelder, 2011).

In addition to primary productivity, also the community structure of planktonic algae responds to variations in C, N, and P concentration in surface water (Padisak et al., 2006). The structure of a planktonic algae community can be studied to infer its nutritional conditions. For example, *Cyanophyta* and *Chlorophyta* have been found to always dominate in eutrophic freshwaters in summer (Rojo et al., 2000; Paerl and Huisman, 2008). The responses of planktonic algae primary productivity and community structure to different nutritional conditions have been studied in rivers, lakes, and reservoirs; however, previous studies have mainly focused on planktonic algae at the phylum level (Xu et al., 2017). Examining the response of single planktonic algae species to nutrient elements during community succession may help in clarifying their outbreak mechanisms (Riebesell et al., 1993; Pan et al., 2016).

The Shawan Karst Test Site, Puding, SW China is a large simulation test site having strictly controlled watershed boundaries and including five different land use patterns (Chen et al., 2017; Zeng et al., 2017). Thanks to the characteristics of this site, it is possible to quantitatively study the effect of land use patterns on the growth of planktonic algae in its manmade spring-fed pond systems (Chen et al., 2017; Zeng et al., 2019). Aquatic photosynthesis has been found to be C-limited in such pond ecosystems (Chen et al., 2017; Zeng et al., 2019). In this study, we will focus on the effects of N, P, and C on the primary productivity and the community composition dynamics of planktonic algae in surface waters (i.e., ponds) under various land use patterns. We hypothesize the following. (1) Land use patterns influence the input of C, N, and P (especially $\text{CO}_{2(\text{aq})}$, NO_3^- , PO_4^{3-}) to surface waters (ponds in this study) in karst areas, as reflected in the unique spatiotemporal hydro-physiochemical characteristics. (2) The phytoplankton algae primary productivity and species composition respond differently to these distinct nutrient characteristics.

2. Materials and methods

2.1. Description of the study site

The Shawan Karst Test Site ($26^{\circ}14' - 26^{\circ}15' \text{N}$, $105^{\circ}42' - 105^{\circ}43' \text{E}$, 1200 m in elevation) is located in the Puding Comprehensive Karst Research and Experimental Station, Puding County, Guizhou Province, China (Fig. 1). The area is characterized by a humid subtropical monsoon climate. The annual mean air T is $\sim 15.1^{\circ} \text{C}$ and the average annual precipitation is 1315 mm, $\sim 80\%$ of which occurs during the rainy season (May–October) (Yang et al., 2012; Zeng et al., 2017). Five concrete tanks were installed at the site to simulate five different patterns of land use: bare-rock, bare-soil, cultivated, grass, and shrub-lands

(Fig. 1b). Each concrete tank was 20 m long, 5 m wide, 3 m deep, and coated with epoxy resin to avoid the influence of possible concrete erosion on the tank hydrochemistry (Zeng et al., 2017). Each tank was partially filled with 2 m of dolomitic limestone gravel, followed by 0.5 m of soil. In the bare-rock land tank, however, no soil was added. The bare-soil land (tank) was filled with both soil and dolomitic limestone gravel, but did not contain any plants. In the cultivated land, instead, we planted corn (growing season: April–August) and applied a compound fertilizer in mid-March. Alfalfa and Roxburgh roses were planted into the soils of the grass and shrub lands, respectively, in January 2014 (Hu et al., 2018). A lateral drainage hole was present in each tank: it simulated a natural karst spring (1S–5S, see Fig. 1b) which fed an artificial pond (1P–5P). The pond, which simulated a lake or reservoir, was 3 m long, 0.5 m wide, and 0.5 m deep (Fig. 1c). Thus, each tank and its connected pond system (Fig. 1b, c) simulated a single land use catchment with precisely known, identical dimensions. All the simulated catchments had the same climatic and geological conditions (Chen et al., 2017; Zeng et al., 2017; Hu et al., 2018; Zeng et al., 2019). Water containing planktonic algae was collected from a nearby karst surface river and poured into the ponds on October 31, 2017. The planktonic algae were hence left to grow in the pond waters. Our monitoring, sampling, and analyses began in January 2018, and the experiment lasted until December 2018, when locally dominant submerged plants (i.e., *Spirogyra*, *Hornwort*, and *Charophyta*) were transplanted into each spring-fed pond.

2.2. Field monitoring

The fieldwork was carried out on one sunny day at the end of each month, so to reduce weather-related effects. T, pH, electrical conductivity (EC, 25°C), and dissolved oxygen (DO) were monitored by using a multi-parameter water quality probe (Wissenschaftlich-Technische Werkstaetten Technology Multiline 350i, Germany). Before using it, we calibrated the parameter sensors as follows: pH = 4.01 and 6.86, EC = $1412 \mu\text{S}/\text{cm}$, DO = 100% air. The measuring accuracies of the device for the pH, T, DO, and EC were 0.01, 0.01°C , $0.01 \text{ mg}/\text{L}$, and $0.01 \mu\text{S}/\text{cm}$, respectively. Moreover, DIC (chiefly in the form of HCO_3^-) was titrated in the field by using an Aquamerck alkalinity test kit (Merck, Germany), with an estimated accuracy of $0.05 \text{ mmol}/\text{L}$ (Zeng et al., 2017). The $\text{CO}_{2(\text{aq})}$ concentration was calculated by software Phreeqc and using field measured temperature, pH, and alkalinity.

2.3. Sample collection and laboratory measurements

A syringe was used to draw water from the pond; then, the water samples were filtered through $0.45\text{-}\mu\text{m}$ Millipore filters and put into 100-mL acid-washed glass bottles and 20-mL plastic square bottles (HDPE) for the analysis of anions and cations, respectively. Ca^{2+} concentration was measured with an ICS-90 ion chromatograph, NO_3^- concentration with an inductively coupled plasma optical emission spectrometer (ICP-OES), and PO_4^{3-} concentration by the Mo-Sb colorimetric method (He et al., 2019; Gong et al., 2012).

Water (2 L) was collected and filtered through $0.45\text{-}\mu\text{m}$ filters (Jinpin $47 \text{ mm} \times 0.45 \mu\text{m}$ glass fiber membrane). After filtration, the membrane was frozen; then, we applied 95% hot ethanol for the determination of Chlorophyll *a*, following the procedure of Sartory and Grobbelaar (1984). Qualitative plankton samples were collected directly from the water using a plankton net (mesh size = 0.064 mm) in a ∞ shape, and then placed in 50-mL centrifuge tubes. Moreover, in order to determine the number of cells for each planktonic algae species, we collected 4 L of water and added to them 4% formalin to enhance the specimens' preservation. After returning to the laboratory, this water sample was concentrated to 30 mL, to allow quantitative and qualitative analyses of the planktonic algae. The single specimens were identified at the species/genus level by inverted microscope (Shi et al., 2017; Hillebrand et al., 1999).

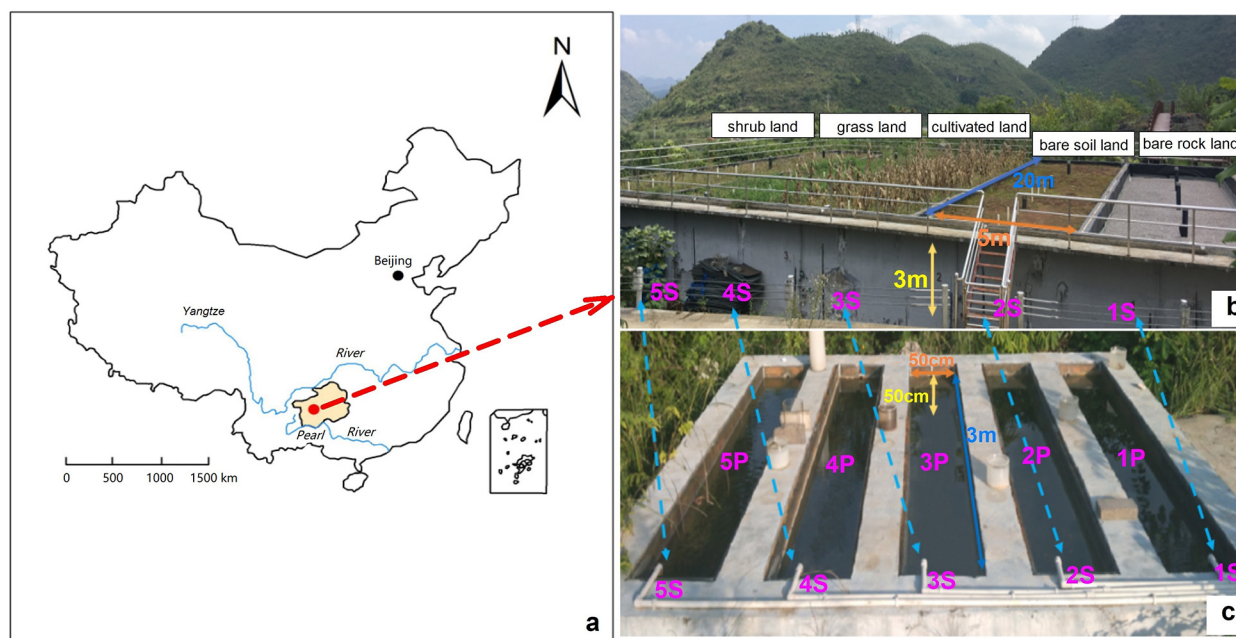


Fig. 1. Location of the Shawan Karst Test Site (a). Five tanks simulated watersheds under different land uses: bare-rock, bare-soil, cultivated (corn), grass (alfalfa), and shrub (Roxburgh roses) lands (b), and the corresponding spring-fed ponds (1S-1P to 5S-5P) (c).

2.4. Data analyses

The correlations between the hydrochemical parameters, the primary productivity of planktonic algae (Chlorophyll *a* and/or algae density), their community structure, and species composition were depicted using SigmaPlot 12.5.

3. Results

3.1. Physiochemical features of the springwaters

The physiochemical features of the five springs (1S–5S) showed both monthly and seasonal variations (Fig. 2). The *T* ranged between 5.4 and 24.9 °C, the lowest and the highest occurring in February and July, respectively (Fig. 2a). The pH ranged between 7.30 and 8.42, reaching its lowest value in June (Fig. 2b); furthermore, the pH values showed a general decrease from 1S (bare-rock land) to 2S (bare-soil land), 3S (cultivated land), 5S (shrub land), and 4S (grass land) (Fig. 2b). Meanwhile, the EC ranged between 201.2 and 515.3 $\mu\text{S}/\text{cm}$ and showed a general decrease from 4S to 3S, 5S, 2S, and 1S (Fig. 2c).

The HCO_3^- concentration also showed some variability (Fig. 2d): it ranged between 1.5 and 5.6 mmol/L, and showed a general decrease from 4S to 5S, 3S, 2S, and 1S. Notably, HCO_3^- concentration increased significantly in 3S and 4S during the growth season.

The NO_3^- concentration ranged between 0.05 and 30.34 mg/L (Fig. 2e), reaching its maximum and minimum values in 3S (in March) and in 5S (in January), respectively. Interestingly, the data showed a significant peak in 3S during the growth season. Overall, the NO_3^- concentration showed a general decrease from 3S to 2S, 1S, 4S, and 5S.

The PO_4^{3-} concentration ranged between 0.0011 and 0.0161 mg/L (Fig. 2f), reaching its highest and lowest values in 3S (in May) and in 5S (in December), respectively. A significant peak was noted in 3S during the growth season, while a smaller peak occurred in 2S between April and July. Overall, the PO_4^{3-} and NO_3^- concentrations showed similar trends.

3.2. Physiochemical features of the pond waters

The physiochemical parameters of the waters contained in the five

ponds (1P–5P) showed both monthly and seasonal variations (Fig. 3). Their *T* ranged from 3.8 °C (in February) to 29.7 °C (in June). As for the springs, no significant differences were observed between the water *T* of different ponds (Fig. 3a). However, pond water *T* tended to be lower in autumn and winter than in spring and summer. The pH ranged between 7.40 and 9.50 (Fig. 3b), and it was higher in the pond than in the springwaters during the experimental period. The EC ranged between 181.6 and 467.6 $\mu\text{S}/\text{cm}$ (Fig. 3c) and, due to the precipitation of CaCO_3 (resulting mainly from aquatic photosynthesis), it was lower in the pond than in the springwaters (Chen et al., 2017). The EC generally decreased from 4P to 5P (3P), 2P, and 1P during the experimental period (Fig. 3c). A plateau of EC was observed in 4P between May and August, while the EC values in 1P, 2P and 5P showed a decreasing trend during the same period (Fig. 3c).

The HCO_3^- concentrations ranged between 1.1 and 5.3 mmol/L (Fig. 3d). Changes in this parameter in the pond waters were similar to those observed for the springwaters; however, the HCO_3^- concentration in the pond waters was always lower than in the corresponding springwaters, due to the photosynthetic consumption of aquatic autotrophs in the former (Chen et al., 2017).

The NO_3^- concentrations ranged between 0.01 and 18.41 mg/L, reaching their maximum in 3P (in June) and their minimum in 5P (in August) (Fig. 3e). Additionally, the NO_3^- values were always lower in the pond waters than in the corresponding springwaters, due to the metabolic consumption of aquatic organisms in the former (Zeng et al., 2019).

The PO_4^{3-} concentrations ranged between 0.0013 and 0.0075 mg/L (Fig. 3f). In spring, summer, and autumn, the PO_4^{3-} values in the pond waters were generally lower than in the corresponding springwaters; however, the opposite trend was observed in winter. These results may derive from the decomposition of the organic matter, which should have caused the release of P into the water. Interestingly, a significant peak in PO_4^{3-} concentration was noted in 3P during the growth season; this represents an attribute inherited from the corresponding spring (3S).

3.3. Primary productivity in the pond waters

Primary productivity in the pond waters was characterized based on

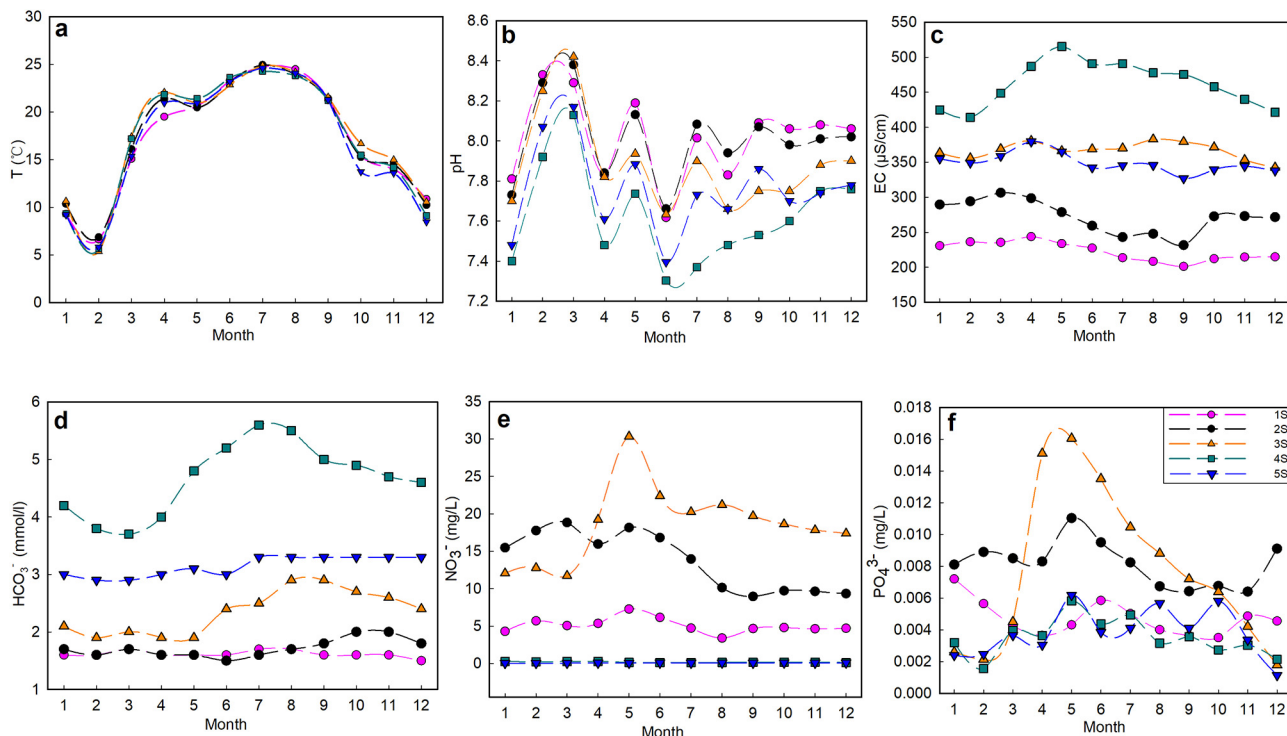


Fig. 2. Monthly variations in the physiochemical parameters (a: T, b: pH, c: EC, d: HCO_3^- , e: NO_3^- , and f: PO_4^{3-}) of the five springs (1S–5S) during the experimental period in 2018.

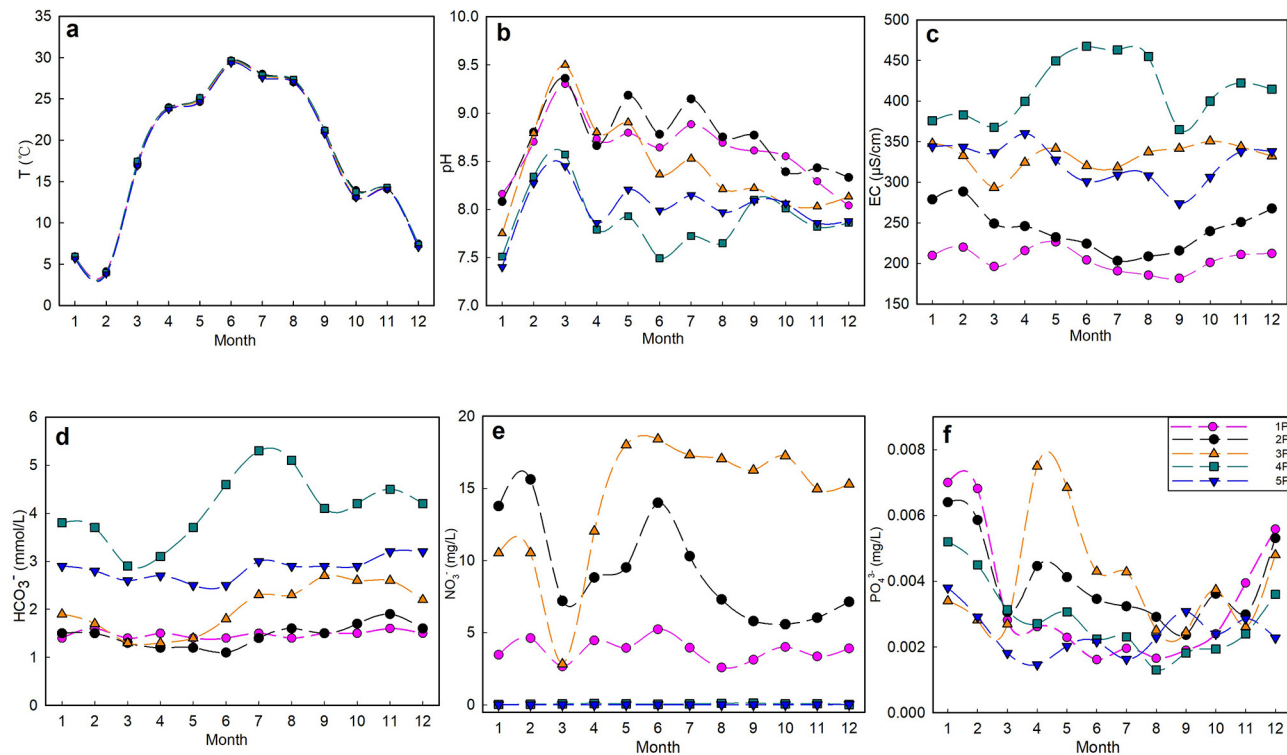


Fig. 3. Monthly variations in the physiochemical parameters (a: T, b: pH, c: EC, d: HCO_3^- , e: NO_3^- , and f: PO_4^{3-}) of the five ponds (1P–5P) during the experimental period in 2018.

Chlorophyll *a* and planktonic algae density (Fig. 4). Chlorophyll *a* ranged between 0.04 and 4.15 mg/m^3 , and was particularly high during the growing season (i.e., March–October), when the T was high. Low values of Chlorophyll *a* were instead observed during the dormant season (i.e., January, February, and December), when the T was low

(Fig. 4a). During the growing period, Chlorophyll *a* increased significantly in all ponds, especially in 3P and 4P in March and April. With time, the Chlorophyll *a* became higher in 1P, 2P, and 3P than in 4P and 5P, especially in June–October (Fig. 4a). The highest Chlorophyll *a* values were registered in 4P in March and April, in concomitance with

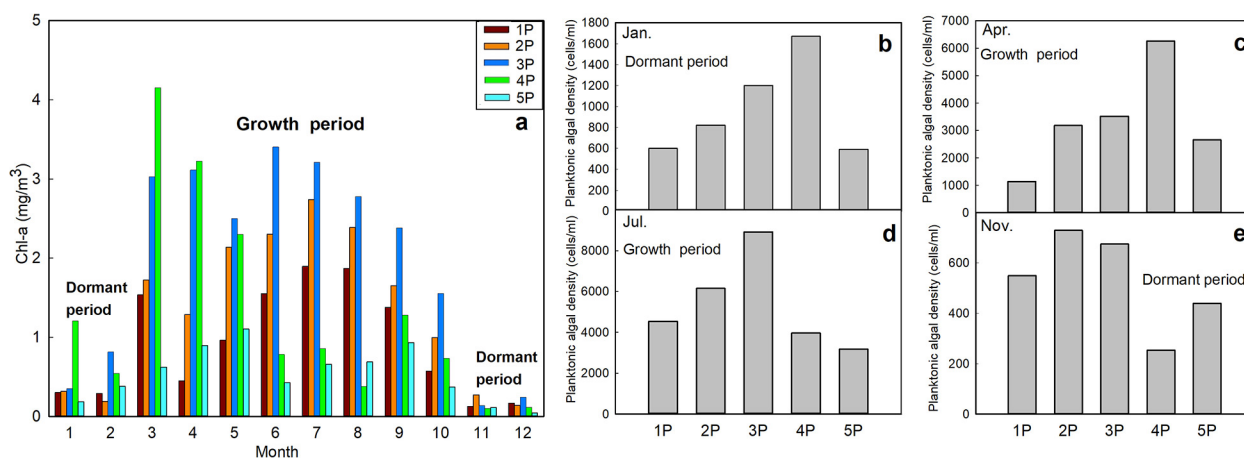


Fig. 4. Spatiotemporal sequences of Chlorophyll *a* (a) and planktonic algae density (b–e) in the five ponds (1P–5P) during the experimental period in 2018.

high HCO_3^- concentrations; moreover, Chlorophyll *a* concentrations were particularly high in 3P in May–October, in concomitance with high NO_3^- and PO_4^{3-} concentrations. Fig. 4 also indicated that, with the growth of planktonic algae, the concentration of Chlorophyll *a* tended to decrease from 3P to 2P, 1P, 4P, and 5P. This decreasing order is consistent with that noted for the NO_3^- and PO_4^{3-} concentrations in the springwaters, which in fact supply nutrients to the ponds.

The planktonic algae density ranged between 254 and 8,919 cells/mL. The highest value was registered in 3P in July, and the lowest in 4P in October. In January, April, July, and October, the order of planktonic algae density was consistent with that of Chlorophyll *a* concentration, indicating that both of these parameters well represented the primary productivity of planktonic algae during the experimental period.

3.4. Planktonic algae abundance and community structure dynamics in the pond waters

The planktonic algae abundance and community structure during the study period are shown in Fig. 5. A total of 62 species (22 of which

were dominant by dominance index, Weaver, 1949) were identified in the five ponds. During the experimental period, the average number of planktonic algae species was 18 in 1P, 22 in 2P, 26 in 3P, 21 in 4P, and 15 in 5P: the highest and lowest number of planktonic algae species were identified in 3P and 5P, respectively. All of the 62 identified species belonged to one of the following: *Cyanophyta*, *Chlorophyta*, *Bacillariophyta*, *Cryptophyta*, *Euglenophyta*, and *Pyrrophyta*. Among these six phyla, *Chlorophyta* and *Bacillariophyta* were the most abundant and dominated the planktonic algae community in the five ponds during the experimental period, while *Cryptophyta* was the less abundant (Fig. 5).

In 1P, the planktonic algae community was dominated by *Bacillariophyta* in winter, and all of the six classes of planktonic algae occurred in this season. During spring, the abundances of *Cyanophyta*, *Chlorophyta*, and *Bacillariophyta* decreased, while those of *Cryptophyta*, *Euglenophyta*, and *Pyrrophyta* increased; nevertheless, *Bacillariophyta* continued to dominate the planktonic algae community throughout the season. During summer, the abundance of *Chlorophyta* increased, while that of *Bacillariophyta* decreased, and *Chlorophyta* dominated the planktonic algae community. In autumn *Cryptophyta* disappeared, while

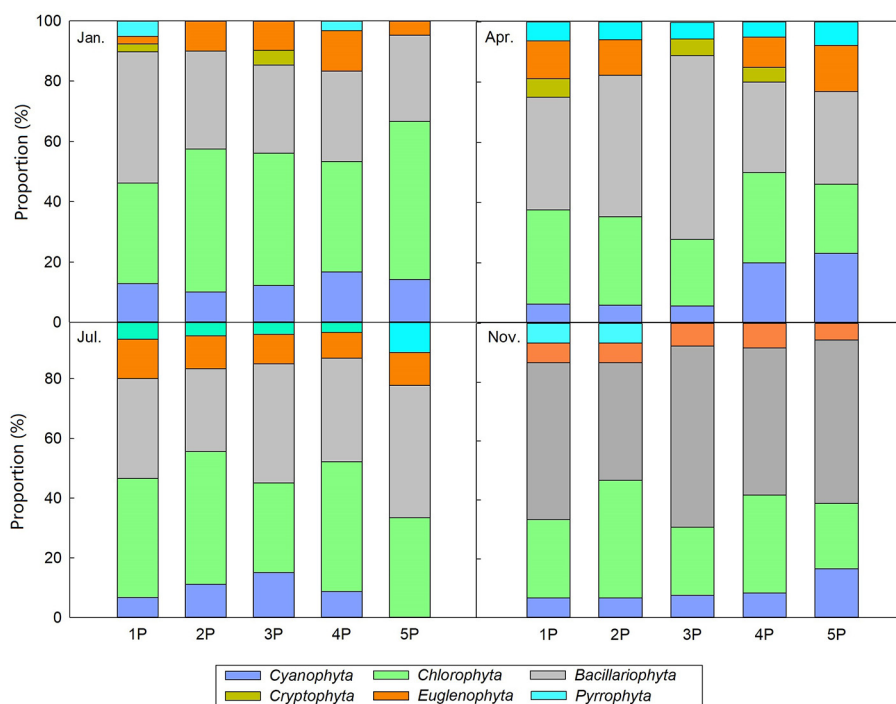


Fig. 5. Variations in the phytoplanktonic algae community structure in the five ponds (1P–5P) during the experimental period.

Table 1
Spatiotemporal succession of dominant planktonic algae species in the five ponds (1P–5P).

Species	1P				2P				3P				4P				5P			
	Jan	Apr	Jul	Nov	Jan	Apr	Jul	Nov	Jan	Apr	Jul	Nov	Jan	Apr	Jul	Nov	Jan	Apr	Jul	Nov
<i>Cyclotella meneghiniana</i>	*	*			*	*			*	*			*	*			*	*		
<i>Fragilaria capucina</i>	*				*				*				*				*			
<i>Synedra acus var</i>	*				*				*				*				*			
<i>Navicula simplex</i>	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
<i>Trachelomonas granulosa</i>	*		*		*		*		*		*		*		*		*		*	
<i>Pandorina morum</i>	*				*				*				*				*			
<i>Aphanocapsa delicatissima</i>	*				*				*				*				*			
<i>Microcystis aeruginosa</i>		*	*			*	*			*	*			*	*			*	*	
<i>Pseudoanabaena</i> sp.				*			*				*	*		*	*		*	*		*
<i>Surirella ovata</i>				*		*	*			*	*		*	*	*		*	*		*
<i>Cymbella pusilla</i>		*	*			*	*			*	*		*	*	*		*	*		*
<i>Ceratium hirundinella</i>		*				*				*			*				*			*
<i>Eudorina elegans</i>						*							*				*			*
<i>Tetraedron minimum</i>		*				*														*
<i>Euastrum ansutum</i>		*	*	*		*	*	*		*	*	*		*	*	*		*	*	*
<i>Chlorella vulgaris</i>		*	*			*				*	*		*	*	*		*	*		*
<i>Merismopedia tenuissima</i>			*			*				*	*		*	*	*		*	*		*
<i>Spirogyra communis</i>			*			*				*	*		*	*	*		*	*		*
<i>Cladophora insignis</i>			*			*				*	*		*	*	*		*	*		*
<i>Melosira granulata</i>				*			*					*			*		*			*
<i>Achnanthes exigua</i>				*			*					*			*		*			*
<i>Scenedesmus bijuba</i>							*					*			*		*			*

*Indicates that it exists in the pond.

Bacillariophyta dominated the planktonic algae community.

In 2P, the planktonic algae community was dominated by *Chlorophyta* in winter and summer. In spring, however, the abundance of *Bacillariophyta* exceeded that of *Chlorophyta*. Moreover, the community was dominated by both *Bacillariophyta* and *Chlorophyta* in autumn (relative abundance = 40% in both cases), while *Cryptophyta* did not occur.

In 3P, the planktonic algae community was dominated by *Bacillariophyta* in spring, summer, and autumn; however, *Chlorophyta* exceeded *Bacillariophyta* in winter.

In 4P, the phytoplankton community was dominated by *Chlorophyta* in summer and winter, while *Bacillariophyta* exceeded *Chlorophyta* in autumn. In spring, *Bacillariophyta* and *Chlorophyta* dominated the community together (relative abundance = 30% in both cases).

In 5P, *Cyanophyta* did not occur during summer, while *Cryptophyta* never occurred during the experimental period.

Table 1 shows the spatiotemporal dynamics of the dominant planktonic algae species succession in the five ponds: a total of 22 dominant species were identified; moreover, the dominant species in each pond showed a significant seasonal succession (Table 1). *Cyclotella meneghiniana*, *Fragilaria capucina*, *Synedra acus* Kützing, *Trachelomonas granulosa*, *Pandorina morum*, and *Aphanocapsa delicatissima* occurred mainly in January and April, while *Microcystis aeruginosa*, *Pseudoanabaena* sp., and some other dominant species occurred mainly in July and November; however, *Navicula simplex* was distributed in all ponds during the whole year. In addition, although some species dominated the planktonic algae communities in the five ponds, their cell number varied considerably among such ponds. For example, *Cyclotella meneghiniana*, *Trachelomonas granulosa*, and *Ceratium hirundinella* showed lower cell numbers in 1P, 2P, and 3P, but higher cell numbers in 4P and 5P. Meanwhile, *Navicula simplex*, *Microcystis aeruginosa*, *Cymbella pusilla*, *Chlorella vulgaris*, and some other dominant species showed higher cell numbers in 1P, 2P, and 3P, but lower cell numbers in 4P and 5P.

4. Discussion

4.1. Effects of land use patterns on the springwaters' hydrochemical features

Land use patterns can affect the soil and groundwater

hydrochemical parameters via plant and microbial activities (Mganga et al., 2016; Zhao et al., 2010; Zeng et al., 2017). In spring, the hydrochemical parameters (i.e., pH, EC, HCO_3^- , NO_3^- , and PO_4^{3-}) of the springwaters varied considerably under different land uses (Fig. 2). These differences indicate that land use patterns have an important impact on karst hydrochemistry.

A previous study (Zeng et al., 2017) has shown the occurrence of significant differences in soil CO_2 under different land uses. After rainfall, soil CO_2 dissolves into soil water in the form of H_2CO_3 . The water reacts with the carbonate rock, creating groundwater with high DIC (including CO_2 (aq), HCO_3^- and CO_3^{2-} ; Liu and Dreybrodt, 2015). The springs under grass and shrub land conditions and with the highest vegetation coverage (i.e., 4S and 5S) showed the lowest pH, as well as the highest EC and HCO_3^- values (Fig. 2b, 2c and 2d). Meanwhile, the springs under bare-rock and bare-soil land conditions and without vegetation coverage (i.e., 1S and 2S) showed the highest pH, as well as the lowest EC and HCO_3^- values (Fig. 2b, 2c, and 2d). The spring under cultivated land conditions (i.e., 3S) changed similarly to the springs under shrub and grass land conditions during the growth period, and to those under bare-rock and bare-soil land conditions during the dormant period (Fig. 2b, 2c, and 2d). These features reflect differences in soil respiration: root biomass and soil microbial activity tend to be high under shrub, grass, and cultivated land conditions (Jia et al., 2010; Costa et al., 2013). Overall, these data indicate that land use affects C dynamics in soil and karst underground water over spatial and temporal sequences (Liu et al., 2007; Yang et al., 2012; Zeng et al., 2017). Fig. 6 further shows the effects of different land uses on the hydrochemistry of groundwater and spring water: soil CO_2 was strongly correlated with the concentrations of Ca^{2+} , DIC, and CO_2 (aq) in both types of water.

Significant differences may exist in the nutrient concentrations of soil and groundwater under different land use patterns (Xiao et al., 2017). Fig. 2e shows that the concentration of NO_3^- in springs decreased from cultivated to bare-soil, bare-rock, grass, and shrub lands during the growth period. The spring concentration of NO_3^- in shrub and grass lands with high vegetation coverage was significantly lower than that in bare-rock, bare-soil, and cultivated lands. This may be because the growth of vegetation consumes a large amount of N in the soil (Xu et al., 2012), or caused by the flow system difference for vegetation can change in-soil flow patterns a lot. The springs under bare-

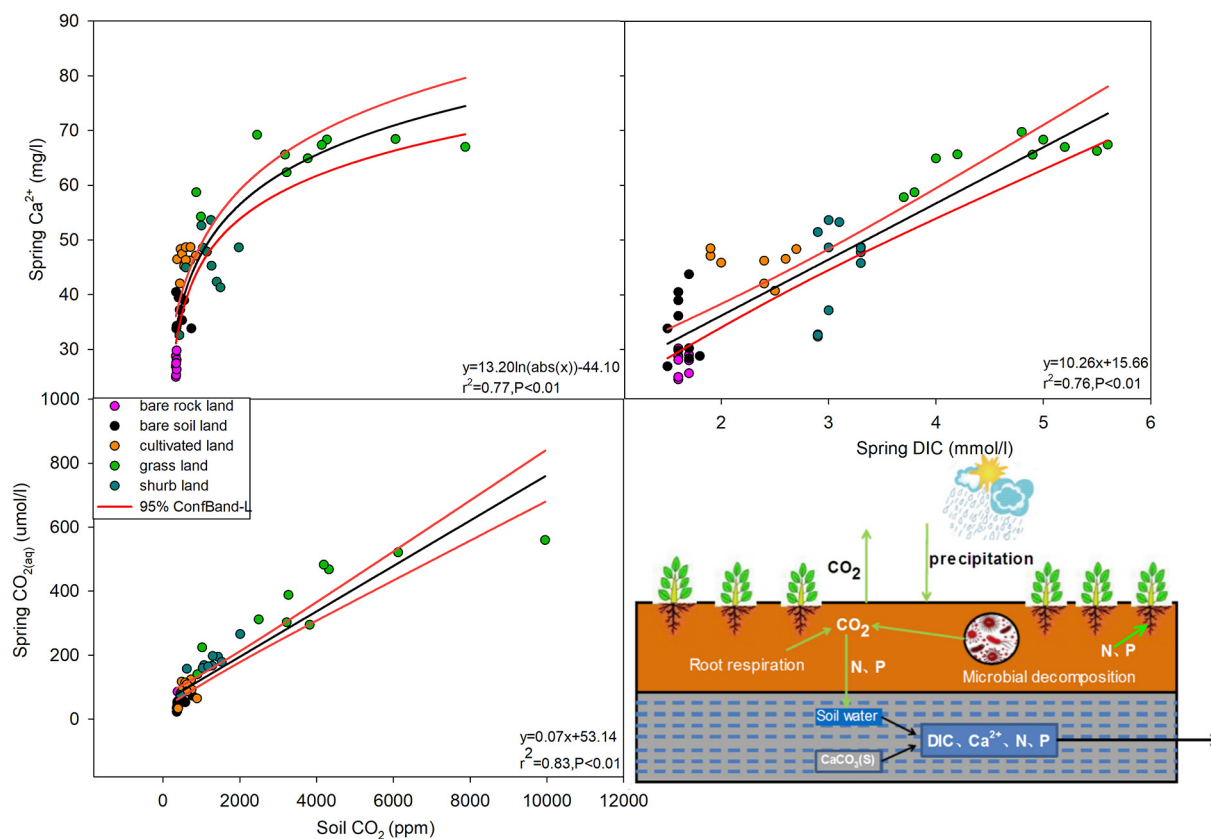


Fig. 6. Effects of different land uses on the hydrochemistry of groundwater, indicated by positive relationships between spring Ca^{2+} and $\text{CO}_{2(\text{aq})}/\text{DIC}$, and soil CO_2 . The “soil CO_2 ” in the bare rock land is the air CO_2 .

rock and bare-soil land conditions may have presented higher NO_3^- concentrations due to the atmospheric deposition of N (Yu et al., 2019). The concentrations of NO_3^- in S3 (under cultivated land conditions) were lower before March (during the dry dormant season), but increased between April–October (during the rainy season). Notably, a compound fertilizer was applied in April for corn cultivation. With the increase of rainfall in May, a large amount of N was leached into the spring (Li et al., 2010). During spring, the variation in PO_4^{3-} and NO_3^- concentrations showed similar features, except under bare-rock land conditions (in which case the PO_4^{3-} concentrations were lower) (Xiao et al., 2017; Gelaw et al., 2015). In summary, our research demonstrated a significant impact of agricultural cultivation on nutrient concentrations in groundwater. This aspect needs to be considered when evaluating the nutrient supply to surface water ecosystems.

4.2. Effects of karst spring hydrochemistry on planktonic algae primary productivity

As discussed above, the hydrochemistry of karst springwaters shows distinct temporal and spatial variations under different land use patterns. Differences in the hydrochemistry of such waters may affect the primary productivity of planktonic algae in the respective surface waters (Jiang et al., 2014; Hayes et al., 2015). Here, we used Chlorophyll *a* and planktonic algae density to characterize the primary productivity (Balch et al., 1992; Chowdhury et al., 2017). After analyzing the level of Chlorophyll *a*, we were able to divide the experimental period into a growth period (March–October, characterized by higher Chlorophyll *a* concentrations) and a dormant period (November–February, characterized by lower Chlorophyll *a* concentrations) (Fig. 4a). Fig. 7 and Fig. 8 show the relationship between the hydrochemical parameters of the springwaters and the primary productivity of planktonic algae in the ponds during the dormant (e.g., in January; see Fig. 7) and growth

(e.g., in July; see Fig. 8) periods. Planktonic algae primary productivity (i.e., planktonic algae density) appeared more sensitive to $\text{CO}_{2(\text{aq})}$ (Fig. 7a) than to NO_3^- (Fig. 7b) or PO_4^{3-} (Fig. 7c) in the dormant period, when DIC concentrations were low (Fig. 2d). However, in the growth period, planktonic algae primary productivity was highly sensitive to NO_3^- (Fig. 8b) and PO_4^{3-} (Fig. 8c). This may be partly related to the seasonal structural succession of planktonic algae having different adaptabilities under different hydrochemical environments (Xu et al., 2015). Our results further show a decrease in *Cyanophyta* and *Chlorophyta*, as well as an increase in *Bacillariophyta*, from January (dormant period) to April (growth period) (Fig. 5). However, *Cyanophyta* and *Chlorophyta* increased and *Bacillariophyta* decreased from April to July (Fig. 5). NO_3^- or PO_4^{3-} showed stronger significant positive correlations with planktonic algae primary productivity than HCO_3^- and $\text{CO}_{2(\text{aq})}$ (Fig. 7), indicating that *Cyanophyta* and *Chlorophyta* were more adaptable than *Bacillariophyta* to the hydrochemical parameters typically verified during hot summers (Beman et al., 2005; Hayes et al., 2015).

4.3. Response of planktonic algae species to changes in C, N, or P concentrations

It has been shown that the primary productivity of planktonic algae can be limited by N, P, or both of them (Howarth and Marino, 2006; Schindler et al., 2016; Tyrrell, 1999); some other studies also consider C as an important limiting factor (Riebesell et al., 1993; Visser et al., 2016; Zeng et al., 2019). However, the above studies have only focused on the total biomass or productivity, ignoring any differences in the response of single planktonic algae species to C, N, and P input to the system (Riebesell et al., 1993; Shi et al., 2017). Here, in order to reveal the distinct response mechanisms of planktonic algae species to spatial and temporal differences in C, N, and P in ponds, we analyzed their cells

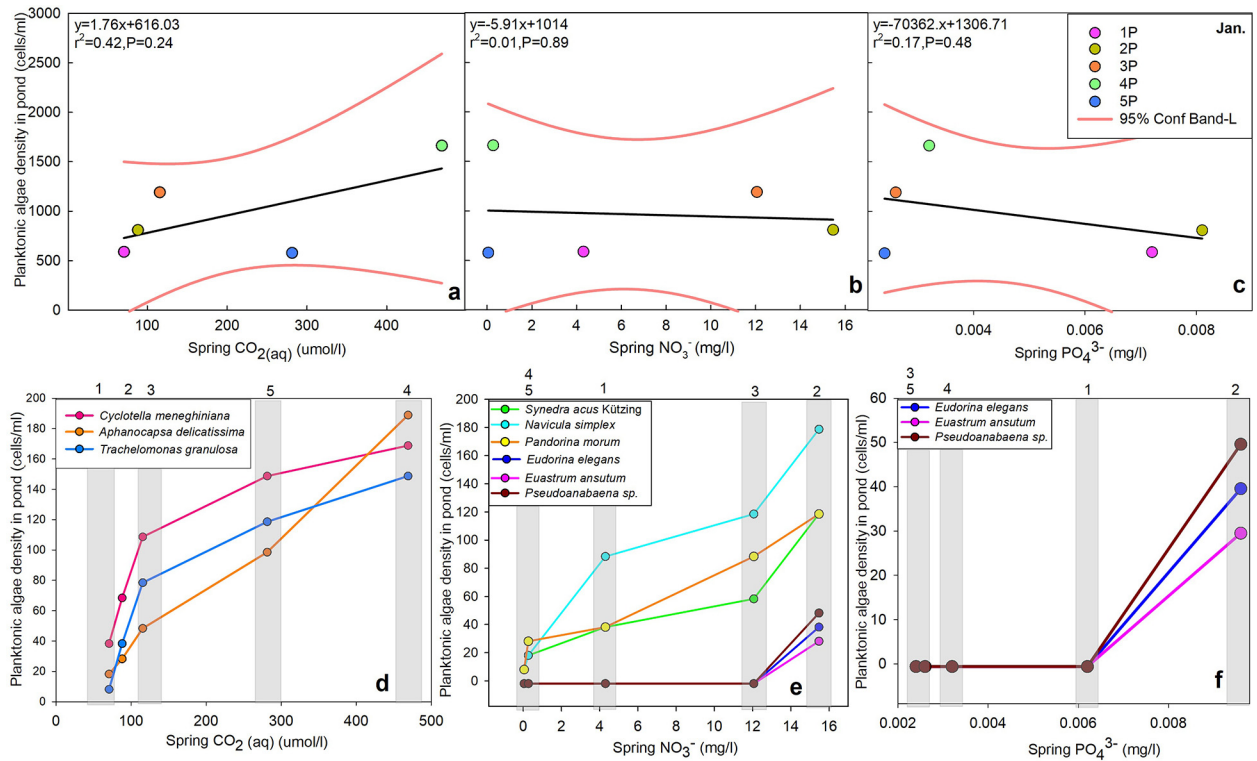


Fig. 7. Variations in planktonic algae density (a–c) and in the cell number of algae species (d–f) in relation to C, N, and P during January (dormant period). Numbers from 1 to 5 represent the spring-pond systems (from 1S-1P to 5S-5P).

separately.

In January, the correlation between the total number of planktonic algal cells and CO_{2(aq)} ($r^2 = 0.42$, $P = 0.42$) was higher than that between the total number of planktonic algal cells and NO₃⁻ ($r^2 = 0.01$, $P = 0.89$) or PO₄³⁻ ($r^2 = 0.17$, $P = 0.48$) (Fig. 7). By

analyzing the elemental preference of planktonic algal cells, we found that *Cyclotella meneghiniana* was sensitive to CO_{2(aq)}; these results support the opinion that this species would clearly respond to changes in CO_{2(aq)} (Shi et al., 2017). We also found that *Aphanocapsa delicatissima* and *Trachelomonas granulosa* may have a response mechanism to CO₂

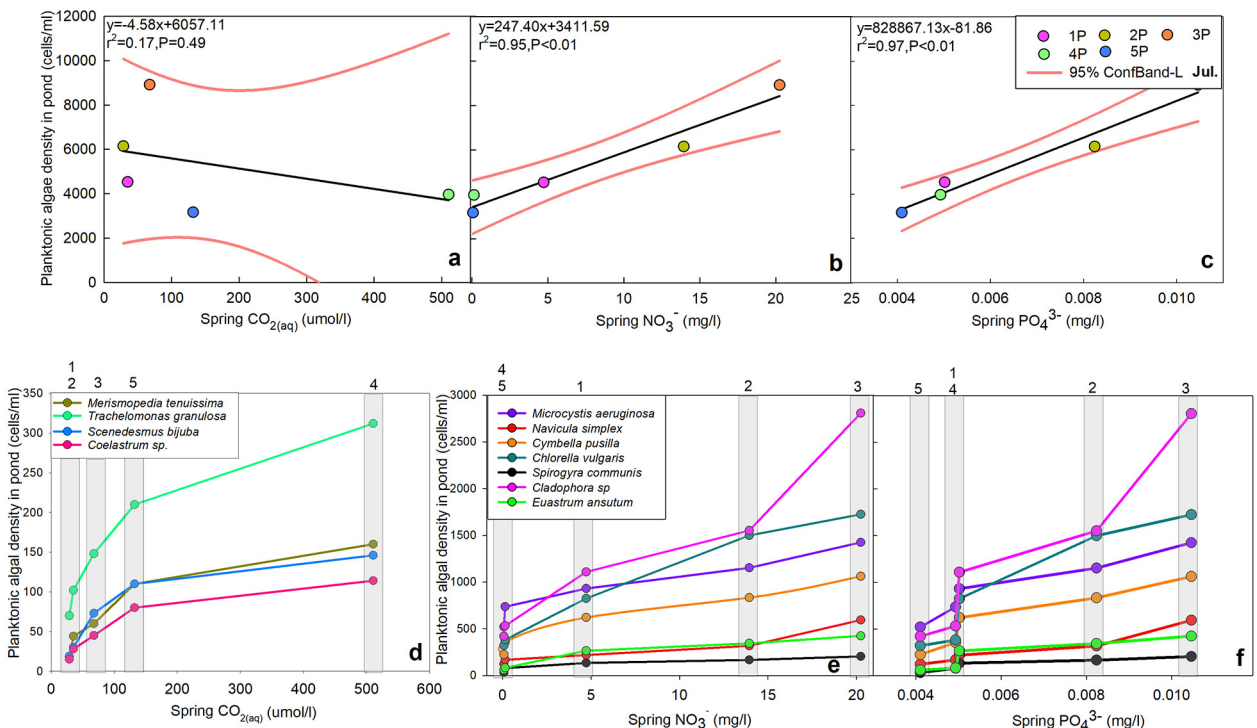


Fig. 8. Variations in planktonic algae density (a–c) and in the cell number of algae species (d–f) in relation to C, N, and P during July (growth period). Numbers from 1 to 5 represent the spring-pond systems (from 1S-1P to 5S-5P).

(aq) similar to that of *Cyclotella meneghiniana*. The results shown in Fig. 7 suggest that *Synedra acus* Kützing, *Navicula simplex*, and *Pandorina morum* were sensitive to the concentration of NO_3^- : their cell numbers increased with NO_3^- concentration, similarly to what was found by Pan and Wang (2016). Furthermore, *Eudorina elegans*, *Euastrum ansutum*, and *Pseudoanabaena* sp. were sensitive to both NO_3^- and PO_4^{3-} . In January, planktonic algal cells seemed to be overall more sensitive to changes in the concentration of CO_2 (aq), rather than in NO_3^- and PO_4^{3-} . However, the response of planktonic algae species to C, N, and P were significantly different in July: the total number of planktonic algal cells had a lower correlation with CO_2 (aq) ($r^2 = 0.17$, $P = 0.49$, Fig. 8a) than with NO_3^- ($r^2 = 0.95$, $P < 0.01$, Fig. 8b) or PO_4^{3-} ($r^2 = 0.97$, $P < 0.01$, Fig. 8c). These results indicated that the growth of planktonic algae is more likely to be limited by N and P during the growing season (Howarth and Marino, 2006; Schindler et al., 2016). Fig. 8 also shows the cell number of several element-sensitive planktonic algae species was one order of magnitude higher in July than in January. Among the planktonic algae sensitive to CO_2 (aq), *Cyclotella meneghiniana* and *Aphanocapsa delicatissima* were replaced by *Merismopedia tenuissima*, *Scenedesmus bijuba*, and *Coelastrum* sp. in July, while *Trachelomonas granulosa* continued to occur. Combining these results with those shown in Fig. 5, we can infer that *Cyanophyta* and *Chlorophyta* tend to grow and bloom in summer under higher T, while *Bacillariophyta* tends to be replaced (Rojo et al., 2000; Paerl and Huisman, 2008). Fig. 8 shows that the planktonic algae species sensitive to N and P tend to coincide, since the N and P concentration gradients presented the same trends in all springs during this period, as evidenced in Fig. 2. We found that *Synedra acus* Kützing, *Pandorina morum*, *Eudorina elegans*, and *Pseudoanabaena* sp., which are sensitive to the concentration of NO_3^- and/or PO_4^{3-} , were replaced by *Microcystis aeruginosa*, *Chlorella vulgaris*, *Cymbella pusilla*, *Spirogyra communis*, and *Cladophora* sp. during this period, while *Navicula simplex* and *Euastrum ansutum* continued to occur. This may be due to changes in T and in the ratio of C, N, and P, which result in the succession of planktonic algae sensitive to different elements (Hayes et al., 2015; Xu et al., 2015; Joehnk et al., 2008). Moreover, our study demonstrates that *Coelastrum* sp., which belongs to *Chlorophyta*, was sensitive to CO_2 (aq), as also found by Shi et al. (2017). Therefore, from the dormant to the growth period, planktonic algae broke out in succession, based on their sensitivities to C, N, and P. Past eutrophication studies have mainly focused on the succession of planktonic algae species (Visser et al., 2016; Skinner et al., 2013). The results of our study indicate that the elemental sensitivity of different planktonic algae species should probably be investigated to further clarify the causes of planktonic algae outbreaks. To conclude, most of the phytoplanktonic algae sensitive to NO_3^- and PO_4^{3-} belong to *Cyanophyta* and *Chlorophyta*, while most of those sensitive to CO_2 (aq) belong to *Bacillariophyta*.

Element stoichiometric ratios have been widely applied to investigate the effects of nutrient limitation on primary productivity in aquatic ecosystems (Rhee, 1978; Klausmeier et al., 2008). In the present study, in order to clarify which element play a key role in the primary productivity of planktonic algae, we considered the values of CO_2 (aq)/TN, CO_2 (aq)/TP, and TN/TP (weight ratio) in the springwaters in both January and July, and examined their correlations to the number of planktonic algal cells in the pond waters (Fig. 9). There is a significant correlation between phytoplankton algae density and CO_2 (aq)/TP ($r^2 = 0.43$, $P = 0.05$, Fig. 9b) in January, indicating that planktonic algae might be more affected by C or P during the low-T dormant period. However, the correlation coefficient of TN/TP ($r^2 = 0.84$, $P < 0.01$, Fig. 9f) was significantly higher than those of CO_2 (aq)/TN ($r^2 = 0.49$, $P = 0.01$, Fig. 9d) and CO_2 (aq)/TP ($r^2 = 0.25$, $P = 0.01$, Fig. 9e) in July, indicating that planktonic algae were mainly limited by N during the high-T growth period.

To further reveal the regulating effects of C, N, and/or P on planktonic algae, the number of cells sensitive to CO_2 (aq), NO_3^- , and PO_4^{3-} in each pond were counted, and their proportion with respect to

the total number of cells were calculated according to the results shown in Fig. 7 and Fig. 8. Fig. 10 shows the concentrations of DIC, CO_2 (aq), NO_3^- , and PO_4^{3-} in the springwaters and the proportion of cells of element-sensitive planktonic algae species in the five ponds under different land uses in January (winter, Fig. 10a) and July (summer, Fig. 10b). It was found that the proportion of planktonic algal cells sensitive to DIC, CO_2 (aq), NO_3^- , and PO_4^{3-} was consistent with their concentration distribution in the five springs in January and July: the primary productivity of planktonic algae seems to be regulated not only by N and P, but also by C (Howarth and Marino, 2006; Schindler et al., 2016; Shi et al., 2017). C limitation occurred mainly during the dormant period, while N and P limitation were readily observed in the growing season. This may be because the number of planktonic algal cells sensitive to N and P during the growing season is much higher than the number of those sensitive to C. In this case, although C-response phenomena occurred, the overall performance of planktonic algae would have depended on N and P availability during the growth period.

We also found that in January (under low T), the proportion of phytoplanktonic algal cells sensitive to NO_3^- and PO_4^{3-} in the ponds accounted for < 30%, while the proportion of cells sensitive to CO_2 (aq) or DIC accounted for 62.71% and 30.54% in the ponds under grass and shrub land conditions, respectively. Therefore, the proportion of phytoplanktonic algal cells sensitive to other elements (possibly Si or Fe, Hutchins et al., 2001) in the ponds under land use patterns other than grass land was > 50%. In the growing period, the proportion of phytoplanktonic algal cells sensitive to NO_3^- and PO_4^{3-} accounted for 90.5%, 95.3%, and 92.4% in the ponds under bare-rock, bare-soil, and cultivated land conditions, respectively; meanwhile, their proportion accounted for only 49.6% and 42.8% in the ponds under grass and shrub land conditions, respectively. The proportion of phytoplanktonic algal cells sensitive to CO_2 (aq) and other elements accounted for 50.4%, 57.2% in the ponds under grass and shrub land conditions, respectively. Furthermore, under grass and shrub land conditions, the pond waters were all characterized by higher CO_2 (aq) and lower NO_3^- and PO_4^{3-} . These results indicate that the springs' hydrochemistry under shrub and grass land conditions, compared to that under bare-rock, bare-soil, and cultivated land conditions, promoted the growth of C- or other elements-sensitive species in the corresponding surface waters; this, in turn, may have contributed to the alleviation of phytoplanktonic algae outbreaks (Jiang et al., 2014; Hayes et al., 2015).

5. Conclusions

Based on the Shawan Karst Test Site, we studied the primary productivity and planktonic algae species composition dynamics in karst surface waters under different land use patterns. Our data indicate that the growth of shrubs and grasses caused a consumption of N and P, as well as the production and addition of abundant CO_2 into the soil. This resulted in lower NO_3^- and PO_4^{3-} concentrations, but also in higher CO_2 (aq) in the springwaters. Meanwhile, the consumption of less N and P and lower soil CO_2 under the bare-rock and bare-soil land conditions resulted in spring waters with higher NO_3^- and PO_4^{3-} concentrations and lower CO_2 (aq). Cultivated lands were characterized by lower NO_3^- , PO_4^{3-} , and CO_2 (aq) values during the dormant period, but higher values during the growth period after the plantation of corn. The fertilization of the cultivated lands had a long-lasting effect on the springs' hydrochemical features.

The relationship between the total number of planktonic algal cells and CO_2 (aq), NO_3^- , and PO_4^{3-} varies with the season. This indicates that planktonic algae primary productivity was regulated by seasonal changes in NO_3^- , PO_4^{3-} , and CO_2 (aq), which might have been caused by the seasonal succession of phytoplanktonic algae species sensitive to different nutrient elements. Further analyses showed that the proportion of phytoplanktonic algal cells sensitive to CO_2 (aq) (dominated by *Cyclotella meneghiniana*) in the ponds under grass and shrub land conditions higher than other ponds in the dormant period. In the growth

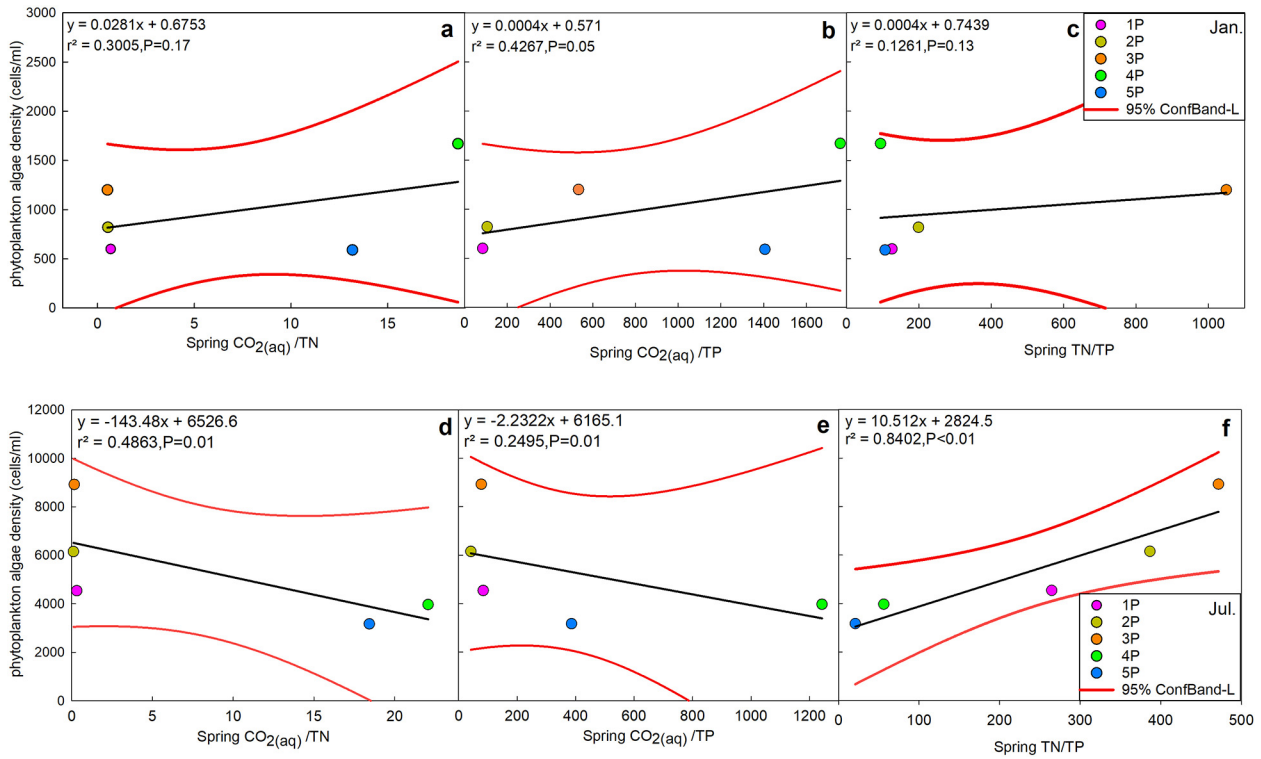


Fig. 9. Correlations between springwater CO_{2(aq)}/TN (a, d), CO_{2(aq)}/TP (b, e), TN/TP (c, f), and planktonic algae density in January and July.

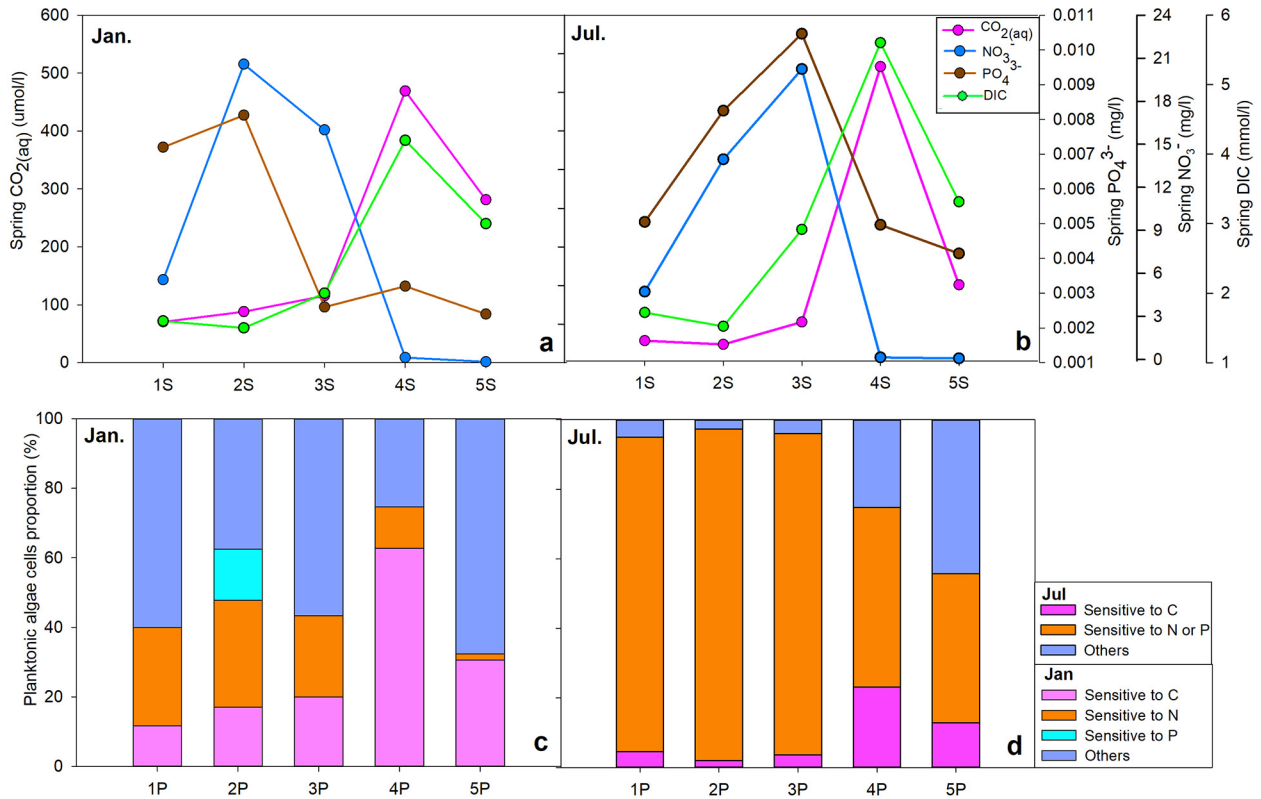


Fig. 10. DIC, CO_{2(aq)}, NO₃⁻, and PO₄³⁻ concentrations in spring (a, b) and proportion of element-sensitive planktonic algal cells in ponds (c, d) under different land uses in January (dormant period) and July (growth period).

period, instead, the proportion of phytoplanktonic algal cells (dominated by *Microcystis aeruginosa*, *Chlorella vulgaris*, and *Cladophora* sp.) was mainly sensitive to NO₃⁻ and PO₄³⁻ in the ponds under bare-rock, bare-soil, and cultivated land conditions. However, the proportion

sensitive to CO_{2(aq)} and other elements (dominated by *Trachelomonas granulosa*) in the ponds under grass and shrub land conditions is higher than other ponds. To conclude, the springs' hydrochemical features under shrub and grass land conditions (i.e., higher CO_{2(aq)}, but lower

NO_3^- and PO_4^{3-}), compared to those under bare-rock, bare-soil and cultivated land conditions, promoted the growth of C-sensitive species in their corresponding surface waters. It is thus predicted that groundwaters with relatively high $\text{CO}_{2(\text{aq})}$ and low NO_3^- and PO_4^{3-} , deriving by natural afforestation in karst areas, may help to alleviate eutrophication and increase C sequestration in the corresponding surface waters, indicating the importance of land-use adjustment in the management of water quality and carbon cycle.

CRedit authorship contribution statement

Qian Bao: Writing - original draft, Software, Visualization, Data curation, Writing - review & editing. **Zaihua Liu:** Conceptualization, Methodology, Supervision, Writing - review & editing. **Min Zhao:** Data curation, Supervision, Formal analysis, Software, Writing - review & editing. **Yundi Hu:** Data curation, Investigation, Writing - review & editing. **Dong Li Curation:** Investigation, Writing - review & editing. **Cuihong Han:** Data curation, Investigation, Writing - review & editing. **Yu Wei:** Data curation, Investigation. **Song Ma:** Data curation, Investigation. **Yi Zhang:** Data curation, Investigation.

Declaration of Competing Interest

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

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