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Limiting factors of planktonic algae' growth in gufu river of three gorges reservoir region, A near-natural river of central China

Jiwen Ge*, Jinpu Gu, Jia Tang, Shuyuan Wu, Guihua Ran, Lu Zeng
Hubei Key Laboratory of Wetland Evolution & Ecological Restoration, Institute of Ecology and Environmental Sciences, China University of Geosciences, Wuhan, 430074, (CHINA)

E-mail : gejiwen2002@aliyun.com

ABSTRACT

From August 2010 to December 2010 and February 2011 to August 2011 (A total of 12 months), we choose 13 sampling points in Gufu River basin which have little human disturbance and rich geological origin phosphorus, to have environmental gradient sampling using field, continuous ways. 156 planktonic algae water samples were collected altogether and more than 2000 valid data were obtained. The data shows that the algae in Gufu River flowing water area are in their growth period, but influenced by restrictive factors, the growth is in deceleration phase; Through correlation analysis, we know that the total nitrogen, nitrate nitrogen and chlorophyll *a* have significant negative correlation, ammonia nitrogen, dissolved oxygen and chlorophyll *a* have significant negative correlation, while total phosphorus, water flow rate, pH and chlorophyll *a* have little correlation between them. The ratio of total nitrogen and total phosphorus in water is 7.06 : 1, The growth of algae reduces the concentration of total nitrogen, but it is also limited by it, their relationship is: $y=0.001203-0.000475x$, $R^2=0.692$, $P=0.00042$ (y stands for the concentration of chlorophyll *a*, x stands for total nitrogen concentration). In terms of nutritive salt, the relationship between environmental restrictive factors and planktonic algae growth is not so obvious.

KEYWORDS

Planktonic algae; Chlorophyll *a*; Limiting factor; Correlation; Gufu River.



INTRODUCTION

In rivers, lakes and reservoirs, algae are the main primary producers^[32], and have a pivotal position in the whole water ecological system. Algae are very sensitive to the surrounding environmental changes, but at the same time, they can build adaptive mechanism quickly, therefore, they have important applications in the water quality indicator, water purification, etc.^[18]. Chlorophyll *a* is easy to be analyzed, and it is an important pigment for algal photosynthesis, so its concentration is usually used to characterize the general content of water algae, as well as judging the water nutritional status. Thus, alga is the key water environmental parameter in limnology and water environmental science^[3,8,16]. Factors which can influence algal growth include light, temperature, pH value, nutrient content of nitrogen and phosphorus, and hydrological conditions of water on its own, etc.^[1,2,5,10,,2535,37]. Then, these factors can be grouped into two types: nutrient and natural environment factors. Nutrient including total phosphorus, total nitrogen, nitrate nitrogen, ammonia nitrogen and silica influence on algal growth leads to interspecific competition advantages^[20], and it's the key component of algae; Natural environment factors also can be divided into two types, climate factors (altitude, temperature, light, etc.) and water quality (dissolved oxygen, water temperature, flow velocity, pH, etc.), which is an important indicator of river ecological system affecting the growth of planktonic algae^[9,25].

The river basin-Gufu River basin chosen as the research subject has special nature and characteristics. On the one hand, Gufu River is a near-natural river which has little human disturbance (there are just a few residences in the middle reaches of Gufu River and is recharged entirely by precipitation and snow-melt water; On the other hand, the river has its source in special place where geological original phosphorus is rich and the ratio between total nitrogen and total phosphorus in Gufu River is almost 7: 1 as our survey. Most researches nowadays focused on those rivers deeply affected by human being and rich in phosphorus, like San Joaquin River^[21], Illinois River^[34], North Bosque River^[29], Daly River^[36] and so on, to study the growth of algae. We tried to find something about how planktonic algae grow under high ratio of total nitrogen and total phosphorus in the river with little human disturbance instead of affected by human being.

Nowadays, algal researches are mostly indoor simulations, through controlling nutritive salt, water temperature, velocity and pH, etc., artificially, to predict the actual algal growth state. Because of the complexity of the natural water ecological system, alga growth is not often determined by a single restrictive factor, but by comprehensive factors of all kinds, and this leads to the indoor simulation defect. The other purpose of this paper is to find how planktonic algae grow in natural state through correlation analysis between chlorophyll *a* of planktonic algae and its limiting factors. Through analyzing the relationship between chlorophyll *a* and environmental factors, we try to explore the restrictive factors affecting the growth of algae in the river basin. The study did an environmental gradient measurement using continuous field sampling of selected points with small human disturbance and geologically rich phosphorus origin in the Three Gorges Reservoir.

MATERIALS AND METHODS

Research area and sampling location

In this study, we choose area of well-confined and small human disturbance in Gufu River (GF) basin (Figure 1) (31.4940°~31.6603°N, 110.8022°~110.9256°E), to set up a total of 13 sampling points between 478~1041 m above sea level, average about 47 meters among each point along the main stream. Gufu River basin is located in the northern Xingshan County of Hubei Province, central China. It originates from Luomadian in Shennongjia Forest District, and also the largest branch of the Three Gorges Reservoir Area of Yangtze River along Hubei Province, which is one of the main origins of Xiangxi brook. The length of the stream is 68 km. The basin area is 1189 km² and the channel average grade is 20‰.

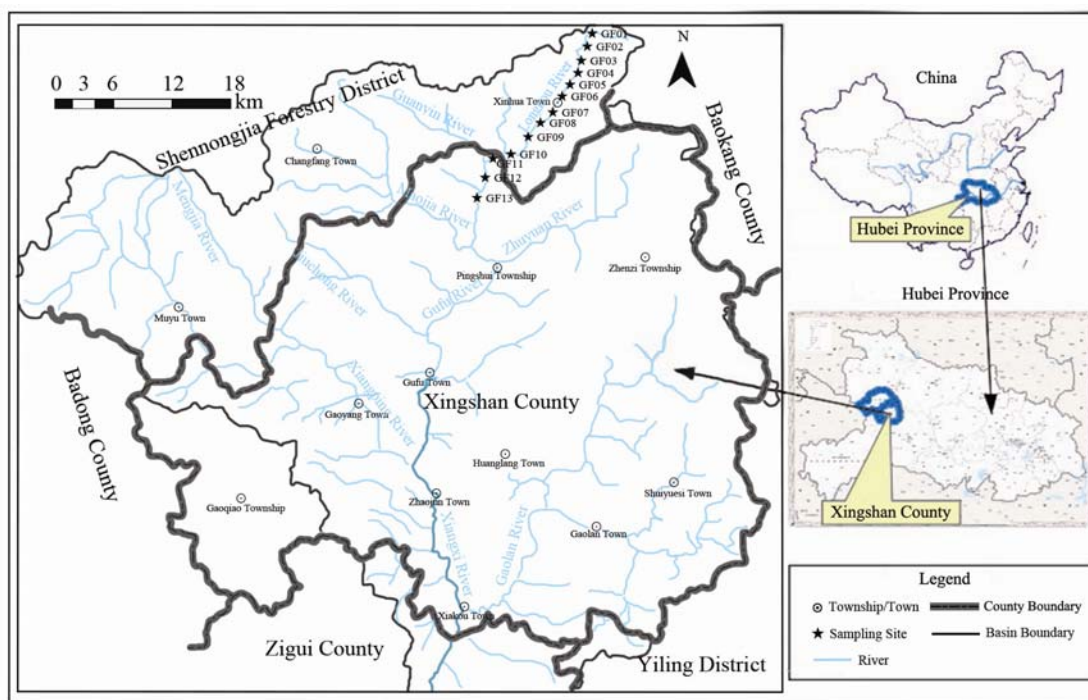


Figure 1 : Distribution of sampling points sites in Gufu River Basin

Sample collection and measurement

The sample acquisition time was from August 2010 to December 2010 and February 2011 to August 2011 (12 months); we selected 13 sampling points in Gufu River basin and took samples in the middle of each month. Sampling was finished in one day; in order to avoid impact such as rainfall, we chose sunny days as far as possible.

In order to find out the restrictive factors of planktonic algae distribution in rich phosphorus river, we selected the following 11 environmental indexes: Chlorophyll *a*, water temperature, water flow velocity, altitude, dissolved oxygen (DO) pH, total nitrogen (TN), total phosphorus (TP), nitrate nitrogen, ammonia nitrogen, and silica. The synchronous measurement projects and the instruments were: altitude, latitude and longitude (GSB-G330, GPS), dissolved oxygen and water temperature (YSI DO200), flow velocity (Global Water FP111), pH (PHB-1-S). Besides, we collected two bottles of water (600 mL for each bottle) in each sampling point back to the laboratory (one with concentrated sulfuric acid acidification to make its pH<2). According to “The Water and Wastewater Monitoring Method (4th edition)” (State Environmental Protection Administration, 2002), we measured the following indexes of correlation: total phosphorus, total nitrogen, nitrate nitrogen, ammonia nitrogen, and silica. The total phosphorus was detected by ammonium molybdate spectrophotometry, total nitrogen by alkaline potassium persulfate digestion-ultraviolet spectrophotometry, nitrate nitrogen by ultraviolet spectrophotometry, and ammonia nitrogen was detected by Nessler's reagent spectrophotometry, silica was detected by ammonium molybdate spectrophotometry.

Measurement of chlorophyll *a*: at the same night, take 2.5L water sample, collected the filter membrane in vacuum flask for cryopreservation after using vacuum filter, and then took them back to the lab for measurement. The laboratory measurement of chlorophyll *a* was in accord with the “Investigation of Lake Eutrophication Specifications (second edition)”^[19].

Data analysis

The analysis of restrictive factors impacted on algal growth was based on monthly weighted average concentration of chlorophyll *a*, total phosphorus, total nitrogen, nitrate nitrogen, ammonia nitrogen, silica, pH and dissolved oxygen, conductivity. Weighted average is the product of the value of each sampling point and its corresponding weight number. Weight number is the ratio between the total

value of each month and the total of all month. Data analysis was achieved through multivariate statistical methods of correlation analysis and regression analysis which was based on the SPSS 19.0 and Excel 2010. All of the above factors including chlorophyll *a*, total phosphorus, total nitrogen, nitrate nitrogen, ammonia nitrogen, silica, dissolved oxygen, pH, flow velocity, altitude, water temperature were used as variables to perform bivariate correlation analysis in SPSS 19.0, then Pearson correlation analysis were chosen as model of correlation analysis and two tailed test as model of significant test, and finally correlation coefficients between chlorophyll *a* and other factors were worked out.

The data of spatial distribution of chlorophyll *a* was used to do curve estimation in regression analysis. The concentration of chlorophyll *a* was taken as the dependent variable and space position variation was taken as the independent variable. Linear, logarithmic and index distribution were selected as the model.

Pearson correlation analysis was used to analyze the relationship between Chlorophyll *a* concentration and other limiting factors of the space.

RESULTS

Spatial variation trend of algae

The average concentration of chlorophyll *a* is 6.54×10^{-4} mg/L ($2.84 \sim 9.09 \times 10^{-4}$ mg/L), which is far lower than that of 16 tributary and 39 reservoirs of Three Gorges Reservoir^[23]. According to the classification standard referred to by Zheng^[39], Gufu River belongs to the poor nutritional type ($\text{Chl } a \leq 3 \mu\text{g/L}$). The average concentration of chlorophyll *a* increases with the sampling point number, among them, GF01 has the lowest concentration of 2.84×10^{-4} mg/L, and GF12 has the highest concentration of about 9.09×10^{-4} mg/L. From GF01 to GF02, the concentration shows the biggest increase at about 3.33×10^{-4} mg/L; Simulation result is shown in Figure 2, among them, logarithmic simulation is the best (its R^2 is 0.609 and P value is 0.00166). Regression equation has a significant statistical significance on 0.002, which passes the significance tests. Linear regression model performs less well than logarithmic model, it has a significant statistical significance on 0.005, while exponential model does not even pass the significance tests ($P=0.0075 > 0.005$). Therefore, through the regression analysis simulation, we can roughly know that algae of Gufu River are in logarithmic phase, but their population growth rate is declining gradually. Through the algae growth curve, we can see that algal growth is in deceleration phase which is gradually restricted by environmental factors.

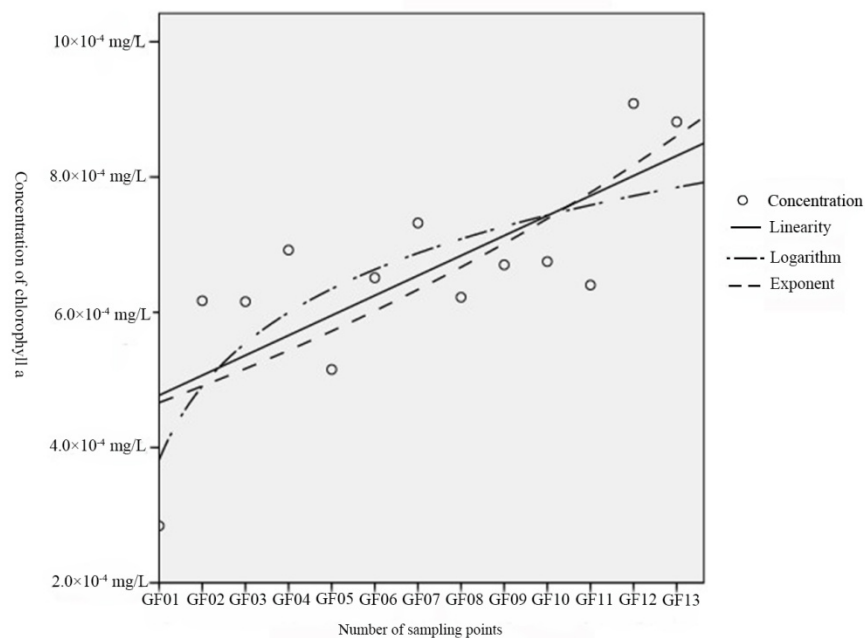


Figure 2 : Spatial change trend and curve simulation of chlorophyll *a* of phytoplankton alga in Gufu River

The correlation between algae and environment restriction factors

Through correlation analysis (TABLE 1) we know that, total nitrogen and nitrate nitrogen are significant negatively correlated with chlorophyll *a*, and their correlation with chlorophyll *a* do not appear to be much different. Total nitrogen has conspicuous positive correlation with nitrate nitrogen, and its correlation coefficient reaches 0.979; Ammonia nitrogen and dissolved oxygen are negatively related with chlorophyll *a*, and they both play an important role in the growth of algae; Silica has positive correlation with chlorophyll *a*; The relation between total phosphorus, water flow rate, altitude, water temperature, pH and chlorophyll *a* are not that obvious, as the correlation coefficients are only -0.109 (total phosphorus), 0.183 (flow velocity), -0.276 (altitude), 0.268 (water temperature) and -0.110 (pH).

TABLE 1: Pearson correlation analysis between chlorophyll *a* of phytoplankton alga and aquatic environment factors of Gufu River

	Total phosphorus	Total nitrogen	Nitrate nitrogen	Ammonia nitrogen	Silica	Dissolved oxygen	pH	Flow velocity	Altitude	Water temperature
Chlorophyll <i>a</i>	-.109	-.832**	-.796**	-.629*	.555*	-.586*	-.110	.183	-.276	.268

**At 0.01 level (double side) significant correlation; *At 0.05 level (double side) significant correlation

DISCUSSIONS

Many domestic and foreign researches^[4,7,12,13,31] showed that, phosphorus is an important factor limiting the algal growth, but through the correlation analysis from our researches the relation between TP and chlorophyll *a* was not obvious in the river, that is to /L), although the concentration of TP which can suit the algal rapid growing condition ranges from 0.020 to 0.230 mg/ say, TP is not that important to the algae. Our study showed the average value of phosphorus in water is 0.159 mg/L (0.150~0.169 mg L^[4]), the average proportion of total nitrogen and total phosphorus reaches 7.06:1. Redfield law thinks that algae cells atomic ratio is C: N: P=106: 16: 1, If the ratio of N: P is more than 16:1, phosphorus is considered to be the restrictive factor, on the other hand, when the ratio of N:P is less than 10: 1, nitrogen is considered to be the restrictive factor; and when converted to mass ratio, when TN: TP >20: 1, it is a performance of the phosphorus deficiency; when TN: TP <13: 1, it is a performance of the nitrogen deficiency^[22]. In this study, the ratio between total nitrogen and total phosphorus was 7.06: 1, which is the performance of nitrogen deficiency, so it means nitrogen is a restrictive factor. This can explain the reason why TP is significantly negatively correlated with chlorophyll *a*.

Zhu *et al.* (2003)^[40] showed that, slow water dynamic condition can play a certain role in algal growth. Within some limits (< 0.4 m/s), increasing water flow velocity is beneficial to the growth of algae^[18]. The average flow velocity of Gufu River is 0.34 m/s. Maximum velocity appears in GF04 sampling point, as the river course here is narrow with the flow velocity of 0.55 m/s; while the minimum velocity appears in GF13 sampling point, as the river course here is wide and flat with the flow velocity of 0.14 m/s; but in general, river flow velocity reasonably maintains at 0~0.4 m/s. Through the Poisson distribution function, we know that within limits of 0~0.4 m/s, the probability can achieve 87.3%. That is to say, this river is suitable for the growth of algae, but the relationship between chlorophyll *a* and the water velocity is very small (the correlation coefficient is only 0.183), which does not show special close relationship between algae growth and velocity.

Temperature is closely tied to the metabolism of algae. Metabolism of algae is increased with the increase of temperature within limits. Date from outdoor survey shows that there is less than 6°C difference between the highest and the lowest temperature in each sampling period. The correlation between chlorophyll *a* concentration and temperature is poor, which shows that water temperature has little effect on the metabolism of algae in each sampling period.

pH is an important ecological factor and is closely related to algal growth, but in Gufu River, the correlation between pH and chlorophyll *a* is not very obvious. Related research^[25] shows that, when pH

value is 8.5, carbonic acid system can reach higher stability and algae grow better. In this river the average pH value is 8.59. The maximum value is 8.77 and the minimum value is 8.37, which fluctuates around 8.5. Increasing of algae population density can gradually increases the pH value^[11], but due to the frequent air exchange between river and mountain, air carbon dioxide is easy to be absorbed into water. Through the carbon balance system and basic ion in water, pH value is reduced, maintaining it in a relatively stable state.

DO is an essential condition for algal growth. Algae need oxygen to grow, multiply and complete metabolism, at the same time, they produce oxygen through photosynthesis. Through data analysis we know, the concentration of dissolved oxygen in the water descends with the process of sampling. Also, through the correlation analysis we know that dissolved oxygen is significantly negatively correlated with chlorophyll *a*. The spatial distribution of chlorophyll *a* shows that, algae are in logarithmic phase, they need oxygen for organic synthesis, so dissolved oxygen in water reduces. Meanwhile, algal photosynthesis and atmospheric oxygen can supply dissolved oxygen for water. But in general, consumption is greater than the supply, which leads to the decline of DO concentration.

Nitrogen is one of the important factors in algal growth, and inorganic nitrogen plays a very important role^[32]. According to the analysis, the vast majority of nitrogen in water exists as nitrate nitrogen (0.531~1.302 mg/L), the nitrate nitrogen accounts for 82.1% of the total nitrogen, while ammonia nitrogen (0.092~0.214 mg/L) accounts for 10.9%. Because the conversion of different forms of nitrogen in water needs a long period of time, several days are often needed for a significant change to appear^[38]. We completed all our samplings in one day, so we ignored the conversion among them. Through correlation analysis we know that, in spatial distribution, chlorophyll *a* is significantly negatively correlated with nitrogen and nitrate nitrogen, and it is negatively correlated with ammonia nitrogen, while the correlation coefficient between total nitrogen and nitrate nitrogen is 0.979, which is very strong.

Algae growth and nitrate nitrogen in water are inseparable, in the researched river, algal growth makes the total nitrogen concentration fall from 1.621 mg/L to 0.639 mg/L, with a 60.5% drop; nitrate nitrogen concentration fall from 1.302 mg/L to 0.531 mg/L, with a 59.3% drop; ammonia nitrogen concentration fall from 0.214 mg/L to 0.108 mg/L, with a 49.6% drop. From the comparison above we can see that, algal growth rely mostly on nitrate nitrogen consumption. Some studies^[28,38] show that, phytoplankton tends to absorb ammonia nitrogen. When algae absorb nitrogen, before nitrogen is synthesized into ammonia nitrogen, nitrate can't be used until it is changed into ammonia salt. But other studies have found out that algae mainly use nitrate nitrogen^[30]. There is no actual mention on what kind of nitrogen on earth do the algae use first. Because the initial concentration of nitrate nitrogen and ammonia nitrogen are not the same, it is difficult to tell which of them is absorbed first. But for the river we study, Algal utilization on nitrate nitrogen (59.3%) is a little more than the utilization of ammonia nitrogen (49.6%).

Soluble silicon is necessary element for diatom^[21]. Diatom accounts for 82.6% of all kinds of planktonic algae in Gufu River, and the rate of silica to total nitrogen in this river range from 1.44 to 4.18, which shows that Gufu River is suitable for the growth of planktonic algae.

Among all those restrictive factors, total nitrogen and chlorophyll *a* has the strongest correlation, their correlation coefficient reaches 0.832. Total nitrogen has conspicuous positive correlation with nitrate nitrogen and dissolved oxygen (their correlation coefficients are 0.979 and 0.685, respectively), and it has a positive correlation with ammonia nitrogen (correlation coefficients is 0.620). So through the determination of total nitrogen content in the water, we can roughly know the content of chlorophyll *a* of algae. When putting the concentration of chlorophyll *a* and total nitrogen together for statistical analysis, we obtain the following regression equation, the corresponding R^2 value and significant coefficient:

$y = 0.001203 - 0.000475x$, $R^2 = 0.692$, $P = 0.00042$ (y stands for the concentration of chlorophyll *a*, x stands for the concentration of total nitrogen).

From the preliminary study we know that, environment at the original river with high phosphorus and low nitrogen did not cause eutrophication^[14], while eutrophication already exists in

branch water environment with low elevation where phosphorus nitrogen ratio is not high^[15]. The author thinks that, there are many factors influencing the algal growth, as in different areas of the river and under different environmental conditions the effect of various factors are different. In this river basin we found out that, the main factor influencing algal growth is nitrogen deficiency. Due to the small human disturbance, the supply of nitrogen element in water mainly relies on the nitrogen cycle under natural condition; this can avoid the man-made interference to water. The growth of algae is limited by nitrogen concentration which keeps it in a relatively low grow situation. Meanwhile the growth curve basically matches with the logistic growth model, which shows the algal adaptability to water. If it keeps this high phosphorus low nitrogen status, the growth of algae can be basically controlled. Once there is source of foreign nitrogen, algae growth environment will change. We can be sure that, with the increase of foreign nitrogen, algae will bloom at a certain range, to some extent may cause the eutrophication of water body. Foreign nitrogen are basically caused by human beings, such as wastewater discharge and application of pesticide and fertilizer, they increase pollution of point source and non-point source. Just like the branch water environment of low altitude in the three gorges reservoir, man-made interferences increase the content of nitrogen, causing the eutrophication of the water.

CONCLUSIONS

Through the data analysis and simulation, we have a preliminary understanding on planktonic algae restrictive factors in rich phosphorus river. We also analyze their rationality, which lays the foundation for the research of algae growth under natural condition.

(1) Under natural condition, algae growth have always been influenced by the surrounding environment, that is one or more restrictive factors leading to the algal tolerance limit, due to those factors, algal growth will go slowly to maximum environmental capacity. Through the analysis on the concentration of chlorophyll *a*, we know that algae are in logarithmic phase, and due to the influence of the restrictive factors, algae population growth rate is declining gradually.

(2) Chlorophyll *a* has conspicuous negative correlation with the concentration of total nitrogen and nitrate nitrogen, and it has negative correlation with the concentration of ammonia nitrogen and dissolved oxygen.

(3) The ratio between total nitrogen and total phosphorus is 7.06:1, total nitrogen is significantly correlated with nitrate nitrogen, ammonia nitrogen and dissolved oxygen, among them total nitrogen has the strongest correlation with chlorophyll *a*. Therefore, total nitrogen is the most important restrictive factor in algae growth, their relationship is the following:

$y = 0.001203 - 0.000475x$, $R^2 = 0.692$, $P = 0.00042$ (y stands for the concentration of chlorophyll *a*, x stands for the concentration of total nitrogen).

(4) Through the correlation analysis on chlorophyll *a* and environment restrictive factors, we know that, in natural water, algae growth has a close relationship with the nutrient, while has little relevance to some natural environment limit factors (water flow velocity, pH, dissolved oxygen, etc.). The author suppose, nutrient is directly involved in the synthesis of algae nutrients, its content directly affects the number of algae, and is inseparable with the growth of algae; under natural condition, algae have produced a relatively stable ecological amplitude to adapt to the surroundings, The ecological factors change to some extent do not affect the growth of algae; therefore, in terms of the nutrient, the relationship between environment restriction factor and algae growth is not that obvious. Hence, when studying the relationship between algae and the environment, we should not be limited to indoor simulation. Habitat conversion will make algae adjust ecological amplitude accordingly, which makes it unable to make the right prediction under natural state.

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