

# THE PHOTOSYNTHETIC PHYSIOLOGY AND GROWTH RESPONSE OF TWO ALGAE SPECIES, *MICROCYSTIS AERUGINOSA* AND *SCENEDESMUS QUADRICAUDA*, TO DIFFERENT NITROGEN FORMS AND CONCENTRATIONS

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**Abstract.** Organic nitrogen and inorganic nitrogen were the main nitrogen sources in the north and south of Dianchi Lake, respectively. Thus, the effect of the different concentration (0.5, 1.0, 2.0, 5.0, 10, 20, 50 mg/L) of ammonium nitrogen (NH<sub>4</sub><sup>+</sup>-N) and alanine on growth of *Microcystis aeruginosa* and *Scenedesmus quadricauda* was investigated. In NH<sub>4</sub>Cl vitro co-culture, *M. aeruginosa* grew better than *S. quadricauda*, and its chlorophyll *a* increased to 2893.94 ug/L in 2 mg/L NH<sub>4</sub>Cl group. In vitro alanine treatments, *S. quadricauda*'s chlorophyll *a* was measured at 5034.34 ug/L in 50 mg/L alanine, which was higher than *M. aeruginosa*. *M. aeruginosa* in NH<sub>4</sub>Cl vitro monocultures showed better cell structure in 20 mg/L NH<sub>4</sub>Cl, and its chlorophyll *a* was higher than that of the corresponding concentration of alanine. The photosynthetic activity (F<sub>v</sub>/F<sub>m</sub>), the maximum electron transfers rate (ETR<sub>max</sub>), and the saturated light intensity point (I<sub>k</sub>) of *M. aeruginosa* increased with the ammonium concentration, and decrease with the alanine concentration, indicating that *M. aeruginosa* can tolerate higher concentrations of ammonium chloride. In high concentrations of NH<sub>4</sub>Cl, *S. quadricauda*'s cell was seriously damaged, but of alanine alone, it was intact. F<sub>v</sub>/F<sub>m</sub>, ETR<sub>max</sub>, and I<sub>k</sub> of *S. quadricauda* increased with the alanine concentrations, showing that *S. quadricauda* makes better use of organic nitrogen.

**Keywords:** ammonium, alanine, chlorophyll *a*, fluorescence characteristic, photosynthesis, cyanobacterial blooms

## Introduction

Nitrogen and phosphorus are key factors of eutrophic freshwater bodies, and the increase in concentrations is generally considered to be the radical cause of cyanobacteria blooms (Conley et al., 2009; Neil et al., 2013; Li et al., 2014; Wang et al., 2015). Outbreaks of cyanobacteria bloom have not been sufficiently prevented, and controlling the total concentration of nutrients is not effective in alleviating the bloom of cyanobacteria. In addition, nutrient morphology also has a corresponding impact on the growth, distribution and community structure of algae (Xu et al., 2019). Nitrogen in natural freshwater body can exist in many forms, for example, dissolved nitrogen not only exists in the form of soluble inorganic nitrogen such as nitrate nitrogen (NO<sub>3</sub><sup>-</sup>-N), nitrite nitrogen (NO<sub>2</sub><sup>-</sup>-N), ammonium nitrogen (NH<sub>4</sub><sup>+</sup>-N) but also in the form of soluble organic nitrogen such as amino acid, urea, amide, hypoxanthine, and guanine. The bioavailability of the nitrogen affects the absorption rate and utilization efficiency of algae. The utilization characteristics of different forms of nitrogen are closely related to the species of algae. It is generally believed that NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N are the main forms of nitrogen utilized by phytoplankton (Zhang et al., 2011). However, the presence of NH<sub>4</sub><sup>+</sup>-N in water

may inhibit the uptake of  $\text{NO}_3^-$ -N by cyanobacteria (Zhu, 2007). The high concentration of  $\text{NH}_4^+$ -N has a certain toxic effect on algae (Tang et al., 2008; Zhang et al., 2011). The main form of organic nitrogen is an amino acid, which comes from phytoplankton. Algae regeneration and death degradation are intertwined, increasing of biomass and organic matter content in the water. In the process of algal bloom, some algal death degradation occurs, which lead to an increase of dissolved organic nitrogen in freshwater (Yu et al., 2016). Therefore, understanding the relationship between amino acids and algal growth is necessary to further understand the ecological relationship between the change of organic matter concentration and algal population. However, some studies focused on the influence of inorganic nitrogen, mainly nitrate and orthophosphate, on algae and algal community structure, and extrapolated the relationship between the change in total concentration and algae growth proposing that the occurrence of cyanobacteria bloom can be controlled by limiting the content of total nitrogen and phosphorus (Wu et al., 2009; Zhu et al., 2018; Peng et al., 2018). However, through investigation of the succession of phytoplankton community in Dianchi Lake, *Microcystis* of cyanophyta and *Scenedesmus* of chlorophyta alternated with seasons. Dianchi lake was divided in 1996 by Haigeng dam into two parts, Caohai Bay in the north and Waihai lake in the south, respectively. The concentration of total nitrogen and total phosphorus in Caohai Bay is higher than that in Waihai lake. *Scenedesmus quadricauda* becomes the dominant species in Caohai Bay in most months of the year, and their biomass is higher than that of *cyanophyta*, while the dominant species is cyanobacteria in Waihai lake (Shi et al., 2014; Hou et al., 2018). That is to say, freshwater bodies with high nitrogen and phosphorus concentrations are not the only factor for cyanobacteria bloom. The influence of nutrient concentration and form interaction on the growth of *Microcystis aeruginosa* and *Scenedesmus quadricauda* in the freshwater body of Dianchi Lake needs further study.

*M. aeruginosa* and *S. quadricauda* which belongs to cyanobacteria and Chlorophyta respectively, are widely distributed in Dianchi Lake. Both of them belong to non-nitrogen fixing algae, so nutrients are one of the limiting factors for their growth. As an autotroph, the photosynthesis of the two species is closely related to the growth of algae and affects the physiological process (Jia et al., 2011). When the growth of algae is limited by available nitrogen, photosynthesis ability to catch light and energy transfer, and carbon fixation are damaged (Geider et al., 1993). Guo (2015) found that the effect of phosphorus concentration on photochemical efficiency of photosystem II (Fv/Fm) was not significant by analysis of the relation between phosphorus concentration and Fv/Fm of *Cladophora oligoclora* Kütz. The absorption and assimilation of inorganic nitrogen by photosynthetic organisms depends on the energy and carbon skeleton produced by photosynthesis and the metabolic process of nitrogen absorption in chloroplasts and mitochondria of algae, so nitrogen is a necessary ecological factor for energy transfer in photosynthesis (Young and Beardall, 2003). There is a certain relationship between the occurrence of nitrogen sources of different forms and concentrations with photosynthetic activity, photosynthetic efficiency and other parameters (Liu et al., 2019). Therefore, whether the form of the nitrogen source produces stress on algae or whether the ability of algae to resist adversity can be measured by using chlorophyll fluorescence parameters needs further study. Based on these discussions, this study takes *M. aeruginosa* and *S. quadricauda*, which are the dominant species of cyanobacteria and green algae in Dianchi Lake, as representative algal species. Alanine is a common amino acid in natural water, which makes up protein, while the ammonium nitrogen is an inorganic nitrogen widely existing in lake. So this study uses alanine and ammonium chloride as nitrogen sources, to explore the effects of

different formations and concentrations of nitrogen on the growth of algae, and to study the relationship between the fluorescence parameters of algae from different nitrogen sources, and to explore the physiological response of *M. aeruginosa* and *S. quadricauda* to different nitrogen sources.

## Methods

### *Experimental design*

We chose *Microcystis aeruginosa* and *Scenedesmus quadricauda* from the Freshwater Algae Culture Collection of the Institute of Hydrobiology (FACHB-collection, Wuhan, China). These algae were cultured in medium in a light incubator under the condition of 25°C, a dark/light period of 12 h: 12 h, the light intensity of 3000-4000 lx, and shaken twice a day. The medium is BG11 which is composed of NaNO<sub>3</sub>, K<sub>2</sub>HPO<sub>4</sub>, MgSO<sub>4</sub>·7H<sub>2</sub>O, CaCl<sub>2</sub>·2H<sub>2</sub>O, Citric acid, EDTANa<sub>2</sub>, CaCO<sub>3</sub>, H<sub>3</sub>BO<sub>3</sub>, MnCl<sub>2</sub>·4H<sub>2</sub>O, ZnSO<sub>4</sub>·7H<sub>2</sub>O, Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O, CuSO<sub>4</sub>·5H<sub>2</sub>O, Co(NO<sub>3</sub>)<sub>6</sub>·6H<sub>2</sub>O. *M. aeruginosa* and *S. quadricauda* cells were collected in the logarithmic growth period by centrifugation (4000 rpm, 8 min). We rinsed two algae 3 times by N-free medium and then inoculated in a medium for 48 h to exhaust nutrients stored in cells. Then, alanine and ammonium nitrogen were used as nitrogen sources with a 5 g/L concentration of nitrogen and was filtered using a 0.22 µm microporous membrane. The concentration gradient of ammonium nitrogen and alanine in each treatment group was 0.5, 1.0, 2.0, 5.0, 10, 20, 50 mg/L. The experiment was divided into three groups. In the first group, *M. aeruginosa* were separately cultured in alanine and ammonium nitrogen at the above N concentration gradient. In the second group, monocultures of *S. quadricauda* were separately exposed to two nitrogen sources, alanine and ammonium, under the same N concentration gradient. In the third group, *M. aeruginosa* and *S. quadricauda* were cultured together at the above N concentration gradient with respectively ammonium nitrogen and alanine. Before combining *M. aeruginosa* and *S. quadricauda* into a co-culture, we counted the algae cells under the microscope to ensure that the number of algae cells is approximately the same value with 1.7×10<sup>6</sup> cells/ml. In all, each treatment group of the experiment was performed in triplicate. Each treatment group was cultured for 18 days, and 3 ml of algal solution in each treatment group was taken every 2 days for determination of physiological characteristics and chlorophyll *a*. At the end of culture period, the cell morphology of two algal was monitored under a microscope and the morphological pictures were obtained by scope photo 3.0.

### *Analytical methods*

The chlorophyll fluorescence characteristic of algae was determined by phyto PAM (Walz, Germany). Samples need to be dark-adapted for 20 minutes. Then, chlorophyll fluorescence technology is a vivo measurement technology to detect the photosynthetic physiological status and the subtle influence of the external environment on algae which is based on using chlorophyll as an indicator of fitness. The Hansatech Fluorescence Monitoring System PAM (Phyto-PAM, WALZ, Germany) was used to measure the chlorophyll *a*, chlorophyll fluorescence parameter of *M. aeruginosa* and *S. quadricauda* every two days in cultural period. The maximum photochemical efficiency (Fv/Fm) of photosystem II (PSII) is to measure the maximum chlorophyll fluorescence (Fm) of algal cells under dark adaptation. The formula described by Genty et al. (1989) is as follows:

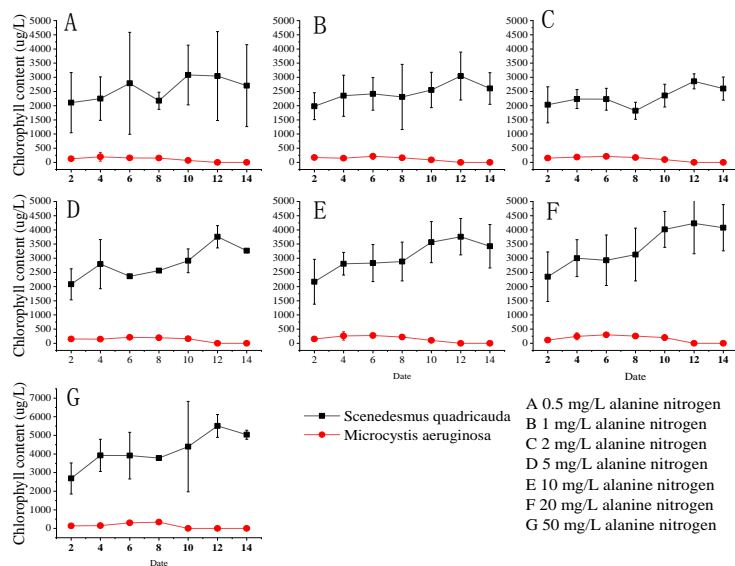
$F_v/F_m = (F_m - F_o)/F_m$ , where  $F_m$  is the maximum fluorescence, which is measured when all reaction centers of PSII are completely closed and all non-photochemical processes are in the minimum under dark adaptation state.  $F_o$  is the initial fluorescence, which is the fluorescence value when all reaction centers of PSII are completely open and all non-photochemical processes are in the minimum under dark adaptation state.  $F_v$  is the maximum variable fluorescence when all non-photochemical processes are in the minimum under dark adaptation state,  $F_v = F_m - F_o$ . According to Ting and Owens (1992), the rapid light curve was measured under nine light intensity gradients (90, 162, 226, 334, 486, 707, 1075, 1586, 2343  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ). The relative electron transfer rate of PSII (ETR) is calculated by the formula as follows:  $r\text{ETR} = \text{Yield} \times 0.5 \times \text{PFD}$ , where Yield is the effective photochemical efficiency of PSII, the coefficient of 0.5 represents that 50% of all absorbed photons are allocated to PSII, PFD is the intensity of photochemical light. The maximum relative electron transfer rate (ETR<sub>max</sub>) and photosynthetic efficiency ( $\alpha$ ) were obtained by fitting the rapid light response curve with original software (8.0) according to the exponential formula  $\text{ETR} = \text{ETR}_{\text{max}} \times [1 - \exp(-\alpha \times \text{PFD}/\text{ETR}_{\text{max}})]$ . The semi-saturated light intensity ( $I_k$ ) reflects the tolerance of the sample to strong light, and the calculation formula is as follows:  $I_k = \text{ETR}_{\text{max}}/\alpha$ . The average chlorophyll *a* and chlorophyll fluorescence parameter of *M. aeruginosa* and *S. quadricauda* were calculated by Microsoft Excel (2010) and Origin 8.0 software.

## Result

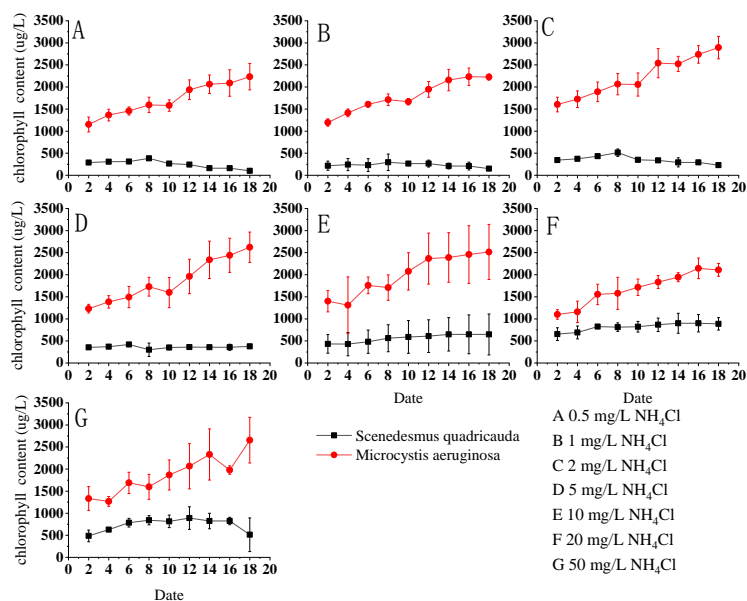
### *Comparison of chlorophyll a content of M. aeruginosa and S. quadricauda in co-culture experiment with separately two nitrogen sources*

The content of chlorophyll *a* of *M. aeruginosa* and *S. quadricauda* in a co-cultured experiment with different concentrations of alanine as nitrogen source is shown in *Figure 1*. The utilization advantage of alanine by *S. quadricauda* is higher than that of *M. aeruginosa*. The content of chlorophyll *a* of *S. quadricauda* was higher than that of *M. aeruginosa*, and increased with alanine concentration during the co-culture period. However, the chlorophyll *a* content of *M. aeruginosa* decreased with time in co-culture experiment. After co-culture in 12 days, the content of chlorophyll *a* of *S. quadricauda* in the group of 50 mg/L alanine was as high as 5034.34  $\mu\text{g/L}$ , followed by that in the treatment group of 20 mg/L alanine, which was 4076.58  $\mu\text{g/L}$ . At the end of co-culture period, the content of chlorophyll *a* in *M. aeruginosa* was 0  $\mu\text{g/L}$ . In the range of 0.5-5 mg/L alanine concentration, the content of chlorophyll *a* of *S. quadricauda* was between 2603.93-2706.78  $\mu\text{g/L}$ . The highest chlorophyll *a* of *M. aeruginosa* was 339.38  $\mu\text{g/L}$ , which was far lower than that of *S. quadricauda*.

The content of chlorophyll *a* of two algae species in the co-cultured experiment with different concentrations of ammonium is shown in *Figure 2*. In different concentrations of ammonium treatment group, the chlorophyll *a* in *M. aeruginosa* was higher than that of *S. quadricauda*, which indicated that the utilization advantage of *M. aeruginosa* to ammonium chloride was higher than that of *S. quadricauda*. The *M. aeruginosa*'s chlorophyll *a* rose with the culture time, while the chlorophyll *a* of *S. quadricauda* showed the opposite trend. After 16 days of co-culture, the chlorophyll *a* content of *M. aeruginosa* in each ammonium treatment group was twice the initial content, and the highest content of chlorophyll *a* of it was 2893.94  $\mu\text{g/L}$  in the 2 mg/L  $\text{NH}_4\text{Cl}$  group. In the entire culture cycle, the highest chlorophyll *a* of *S. quadricauda* was 891.14  $\mu\text{g/L}$ , which was far lower than that of *M. aeruginosa*.



**Figure 1.** Chlorophyll a of *M. aeruginosa* and *S. quadricauda* in co-cultured experiment with alanine nitrogen source. The error bars is standard deviation of Chlorophyll a

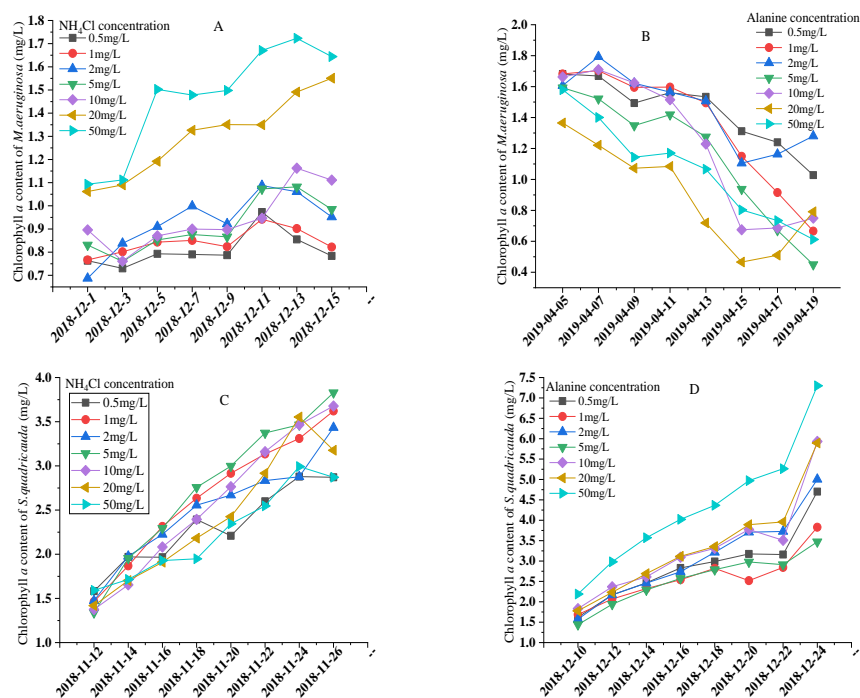


**Figure 2.** Chlorophyll a of *M. aeruginosa* and *S. quadricauda* in co-culture experiment with ammonium nitrogen source. The error bars are standard deviation of Chlorophyll a

### The chlorophyll a content of *M. aeruginosa* and *S. quadricauda* under two nitrogen sources in monoculture experiment

Using alanine and ammonium as nitrogen sources, we cultured the *S. quadricauda* and *M. aeruginosa* respectively. The changing trend of chlorophyll a of the two algae is shown in Figure 3. In the ammonium nitrogen treatment group (Figure 3A), the chlorophyll a content of *M. aeruginosa* increased with time in the high concentration of 10, 20, 50 mg/L ammonium nitrogen treatment group, and the content was higher than

that of other groups. In 0.5, 1, 2, 5 mg/L low concentration ammonium nitrogen treatment groups, the content of chlorophyll *a* of *M. aeruginosa* increased at the initial stage of culture, and decreased gradually after 11 days, which was lower than that of high concentration ammonium group. At the end of the culture period, the chlorophyll *a* content of *M. aeruginosa* was 1.74, 1.54, 1.16 mg/L separately in 50, 20, 10 mg/L ammonium nitrogen group, which was 1.7, 1.5 and 1.3 times higher than that in the initial culture. The chlorophyll *a* content of *M. aeruginosa* was 0.72, 0.73, 0.87, 0.93 mg/L separately in 0.5, 1, 2, 5 mg/L ammonium nitrogen group, which was slightly higher or lower than that in the initial culture. In the alanine treated group (Figure 3B), the chlorophyll *a* content of *M. aeruginosa* decreased with the culture time, and the chlorophyll *a* in each treated group was lower in the end of the experimental phase than that at the beginning phase. In the entire growth period, the *M. aeruginosa*'s chlorophyll *a* in 0.5, 1, 2 mg/L alanine group was higher than that of other high concentration groups. The results showed that the content of chlorophyll *a* of *M. aeruginosa* in low alanine concentration group was higher than that in high concentration group, especially in 20 mg/L and 50 mg/L alanine group, the chlorophyll *a* content of *M. aeruginosa* was lower than that of other treatment groups. Comparison of ammonium nitrogen and alanine treated groups, we discovered that the growth potential of *M. aeruginosa* increased with the high of the concentration of ammonium nitrogen, and the maximum growth potential happened in the treatment of 20 mg/L and 50 mg/L of ammonium nitrogen, but in the treatment of alanine, the growth trend of *M. aeruginosa* decreased with the increase of the concentration of alanine, which indicated that *M. aeruginosa* has better utilization of inorganic nitrogen than organic nitrogen.



**Figure 3.** Chlorophyll *a* of in *M. aeruginosa* and *S. quadricauda* in monoculture experiment with ammonium and alanine separately. *M. aeruginosa* and *S. quadricauda* from the Freshwater Algae Culture Collection of the Institute of Hydrobiology (FACHB-collection, Wuhan, China) were cultured in medium in a light incubator for 2 months. Then these algae were used in the experiment

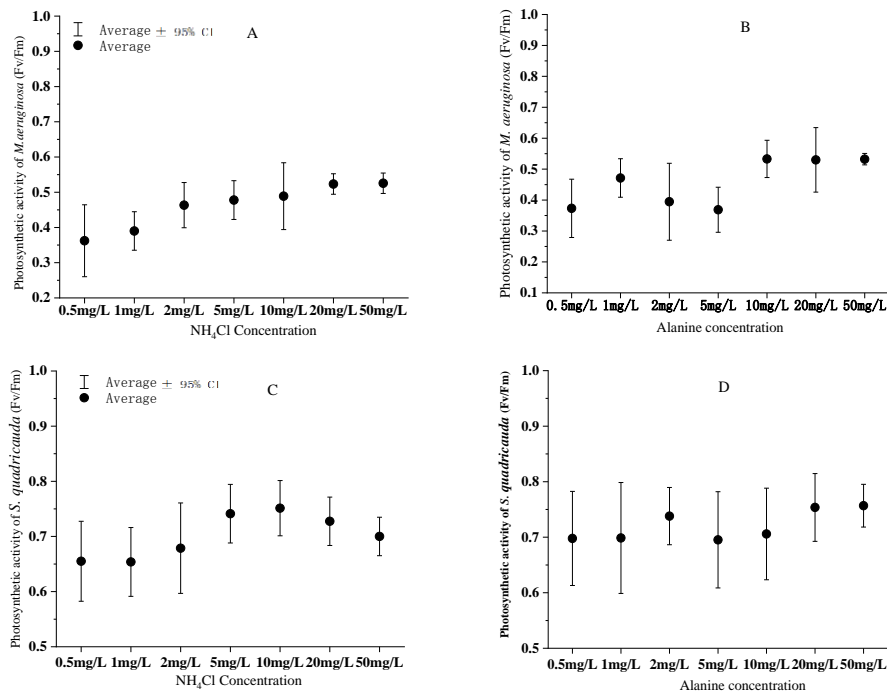
*S. quadricauda* was cultured respectively with alanine and ammonium as nitrogen sources (Figure 3C, D), chlorophyll *a* content is as follows, the growth of *S. quadricauda* was the highest in the treatment group of 5 mg/L and 10 mg/L concentration ammonium, that the chlorophyll *a* increased nearly three times than that in the beginning phase. With the increase of ammonium concentration, the growth of *S. quadricauda* tended to decline. The growth potential of *S. quadricauda* in 1 mg/L and 5 mg/L alanine treatment group was the worst. In 50 mg/L alanine treatment group, the *S. quadricauda*'s chlorophyll *a* increased rapidly and reached to 7.3 mg/L. Comparison to ammonium and alanine treated groups, we found that the chlorophyll *a* of *S. quadricauda* (Figure 3D) in alanine treatment groups was higher than that in the corresponding concentration of ammonium treatment group, which indicated that organic nitrogen (alanine) is better than inorganic nitrogen (ammonium nitrogen) for the growth of *S. quadricauda*. At the end of experimental culture phase, chlorophyll *a* of *S. quadricauda* was the same value of 2.87 mg/L in the treatment group of 0.5 mg/L and 50 mg/L ammonium nitrogen, which were the lowest among all the ammonium treatment groups. The *S. quadricauda*'s chlorophyll *a* was the highest in the treatment group of 5 mg/L and 10 mg/L ammonium, which indicated that 5-10 mg/L ammonium nitrogen was more suitable for the growth of *S. quadricauda*, and 0.5 mg/L extremely low concentration and 50 mg/L extremely high concentration ammonium did not benefit to *S. quadricauda*. In the alanine treatment group, chlorophyll *a* of *S. quadricauda* was the highest in the extremely high concentration of alanine 50 mg/L and 20 mg/L treatment groups, which were 7.30 mg/L and 5.89 mg/L, respectively, which indicated that *S. quadricauda* was more suitable for the high concentration of organic nitrogen.

#### **Comparison of photosynthetic activity of *M. aeruginosa* and *S. quadricauda* under two nitrogen sources**

The photosynthetic activity of *M. aeruginosa* in the treatment group of ammonium and alanine is shown in Figure 4. In the 50 mg/L ammonium group (Figure 4A), the average photosynthetic activity was 0.52 higher than that of other groups, which is the range of 0.47-0.60 in the whole growth period. In the 0.50 mg/L ammonium group, its value was the lowest with the average value of 0.36 and the range of 0.23-0.46. The photosynthetic activity of *M. aeruginosa* rose with the increase of ammonium concentration, indicating that *M. aeruginosa* grew strongly in the high concentration of ammonium. The average photosynthetic activity of *M. aeruginosa* in 10, 20, and 50 mg/L alanine treatment groups (Figure 4B) were the highest, with values of 0.49, 0.52 and 0.53, and the range value of 0.31-0.62, 0.49-0.61 and 0.47-0.60, respectively. The average photosynthetic activity of *M. aeruginosa* in the ammonium group was higher than that in the same alanine concentration group, showing that the growth potential of *M. aeruginosa* in ammonium nitrogen was higher than that in alanine organic nitrogen.

For *S. quadricauda* (Figure 4C, D), the mean photosynthetic activity was the highest (0.74 and 0.75, respectively) in the 5 mg/L and 10 mg/L ammonium treatment group, and the range of values was 0.68-0.80 and 0.66-0.87, respectively, the lower value appeared in the 0.5 mg/L, 1 mg/L and 50 mg/L ammonium group, which indicated that the photosynthetic activity of *S. quadricauda* was very low in very low and high concentration of ammonium nitrogen and the photosynthetic activity of *S. quadricauda* was strong in the medium concentration of ammonium nitrogen. Among the alanine treatment groups (see Figure 4D), the highest average photosynthetic activity of *S. quadricauda* was found in 20 mg/L and 50 mg/L alanine treatment group with the

identical mean values of 0.75 and the range value with 0.66-0.87 and 0.67-0.81, respectively, and these values were higher than that in the corresponding same concentration ammonium treatment group, which indicated that the growth potential of *S. quadricauda* in the high concentration of alanine was higher than that in the high concentration of ammonium.

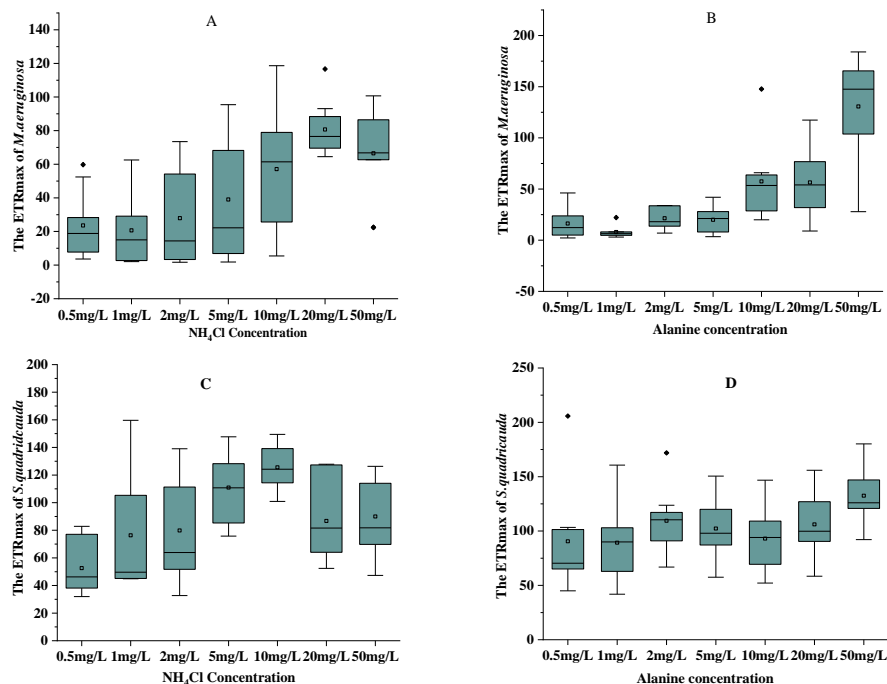


**Figure 4.** The photosynthetic activities of *M. aeruginosa* and *S. quadricauda* under different nitrogen sources. These values were measured every 2 days during the 18-day culture cycle

### **The maximum photosynthetic rate difference of *M. aeruginosa* and *S. quadricauda* in monoculture under two nitrogen sources**

Phyto PAM was used to determine the rapid light response curve of *M. aeruginosa* and *S. quadricauda* under the separate cultivation of two nitrogen sources. The characteristic parameter of the maximum electron transport rate (ETR<sub>max</sub>) is to characterize the photosynthesis efficiency of phytoplankton, and the data is shown in Figure 5. In the high of ammonium concentration such as 10, 20, 50 mg/L treatment group (in Figure 5A), the highest variation of ETR<sub>max</sub> value of *M. aeruginosa* was found in the whole culture period. After 9 days, the ETR<sub>max</sub> of *M. aeruginosa* in the 50 mg/L ammonium group increased gradually, which was higher than that in the other concentration group. And its value of *M. aeruginosa* in 10, 20 mg/L ammonium treatment group increased in the initial period, and then decreased after 9 days. In the alanine treatment group (see Figure 5B), the ETR<sub>max</sub> of *M. aeruginosa* in the high concentration 10, 20, 50 mg/L treatment group was higher than that of the low concentration treatment, and increased gradually with the culture time, reaching the maximum value at 15<sup>th</sup> day, and then decreased gradually. At the end of the experiment, the ETR<sub>max</sub> of *M. aeruginosa* in 50 mg/L alanine group is the highest, followed by 20 mg/L alanine treatment group.



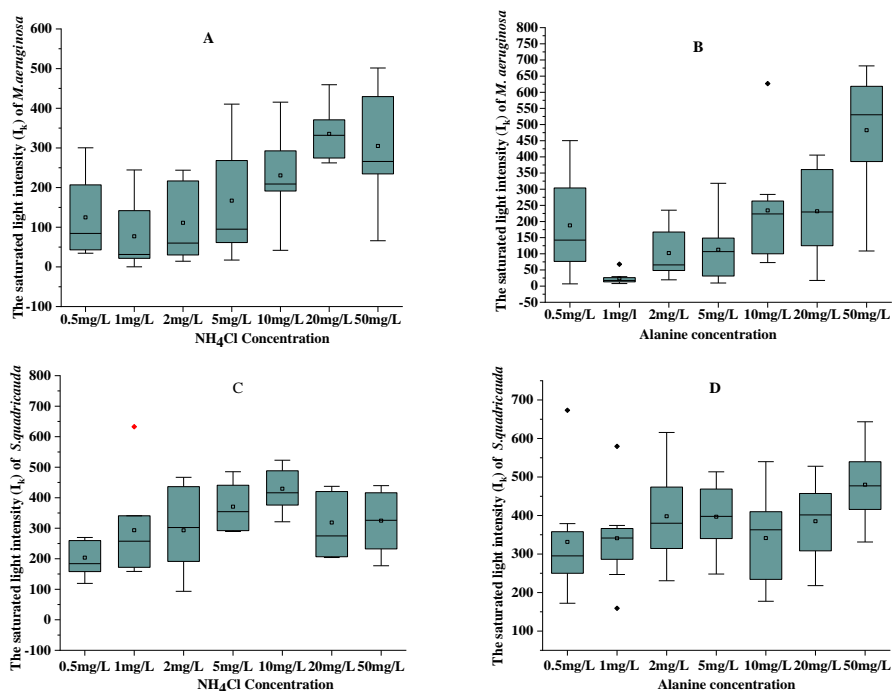


**Figure 5.** The ETRmax of *M. aeruginosa* and *S. quadricauda* under different nitrogen sources. These values were measured every 2 days during the 18-day culture cycle

The ETRmax of *S. quadricauda* treated with ammonium and alanine is as following. In the ammonium treatment group (Figure 5C), except for the 10 mg/L treatment group, the ETRmax of *S. quadricauda* in the other ammonium treatment groups decreased with the culture time in the growth period. The ETRmax of *S. quadricauda* in the 10 mg/L and 5 mg/L ammonium groups (see Figure 5C) was higher than that in the other groups, while that value in the low concentration ammonium (0.5 mg/L and 1 mg/L) treatment group was the lowest. In the alanine treatment group (Figure 5D), the ETRmax of *S. quadricauda* in the 50 mg/L group was higher than that in the other groups, and this value in the low concentration alanine group (0.5 mg/L and 1 mg/L) was the lowest.

### **The difference of saturated light intensity ( $I_k$ ) between *M. aeruginosa* and *S. quadricauda* under two nitrogen sources**

The saturated light intensity ( $I_k$ ) indicates the adaptability of phytoplankton to light intensity. The  $I_k$  values of *M. aeruginosa* and *S. quadricauda* under the monoculture of two nitrogen sources are shown in Figure 6. The median value of  $I_k$  of *M. aeruginosa* in the two nitrogen source treatments grew with the increase of nitrogen concentration (see Figure 6A, B), which showed that *M. aeruginosa* had stronger adaptability to strong light if it was cultured in the high concentration of alanine and ammonium nitrogen. The variation ranges of  $I_k$  of *M. aeruginosa* in the high concentration of alanine group were wide, while in the high concentration of ammonium nitrogen group the variation of this was small. Among the ammonium treatment groups (Figure 6 C), the median value of  $I_k$  of *S. quadricauda* was the lowest in the 0.5 mg/L ammonium group, and the  $I_k$  of *S. quadricauda* in 20 mg/L and 50 mg/L treatment groups was only higher than that in 0.5 mg/L ammonium treatment group, but lower than that in other treatment groups, and the fluctuation range was large.

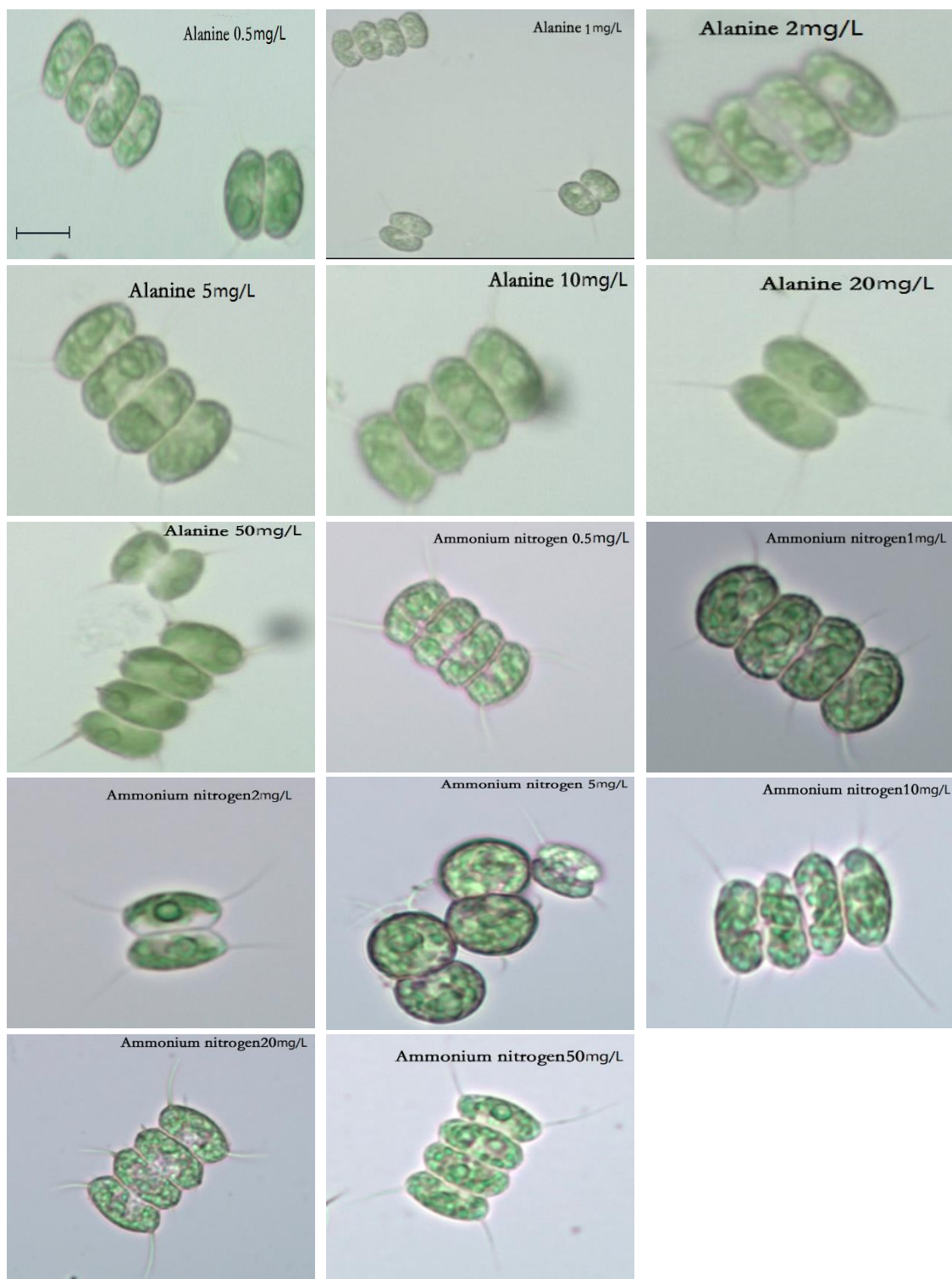


**Figure 6.** The saturated light intensity ( $I_k$ ) of *M. aeruginosa* and *S. quadricauda* under different nitrogen sources. These values were measured every 2 days during the 18-day culture cycle

The  $I_k$  of *S. quadricauda* increased gradually in the alanine treatment group (Figure 6D), and the median  $I_k$  of *S. quadricauda* in 50 mg/L alanine treatment was the highest, and the fluctuation range of this value was very small, which indicated that the high concentration of alanine was better than the low concentration group. The ability of *S. quadricauda* in high concentration of alanine treatment to resist strong light was stronger than that in the low concentration group. In the high concentration (20 mg/L and 50 mg/L) and low concentration (0.5 mg/L and 1 mg/L) of alanine treatment group (Figure 6D), the saturation light intensity ( $I_k$ ) of *S. quadricauda* was higher than that of the corresponding concentration of ammonium nitrogen treatment group, which indicated that the tolerance of *S. quadricauda* to strong light in organic nitrogen source was higher than that of inorganic nitrogen source.

### ***The cell morphology of M. aeruginosa and S. quadricauda under two nitrogen sources***

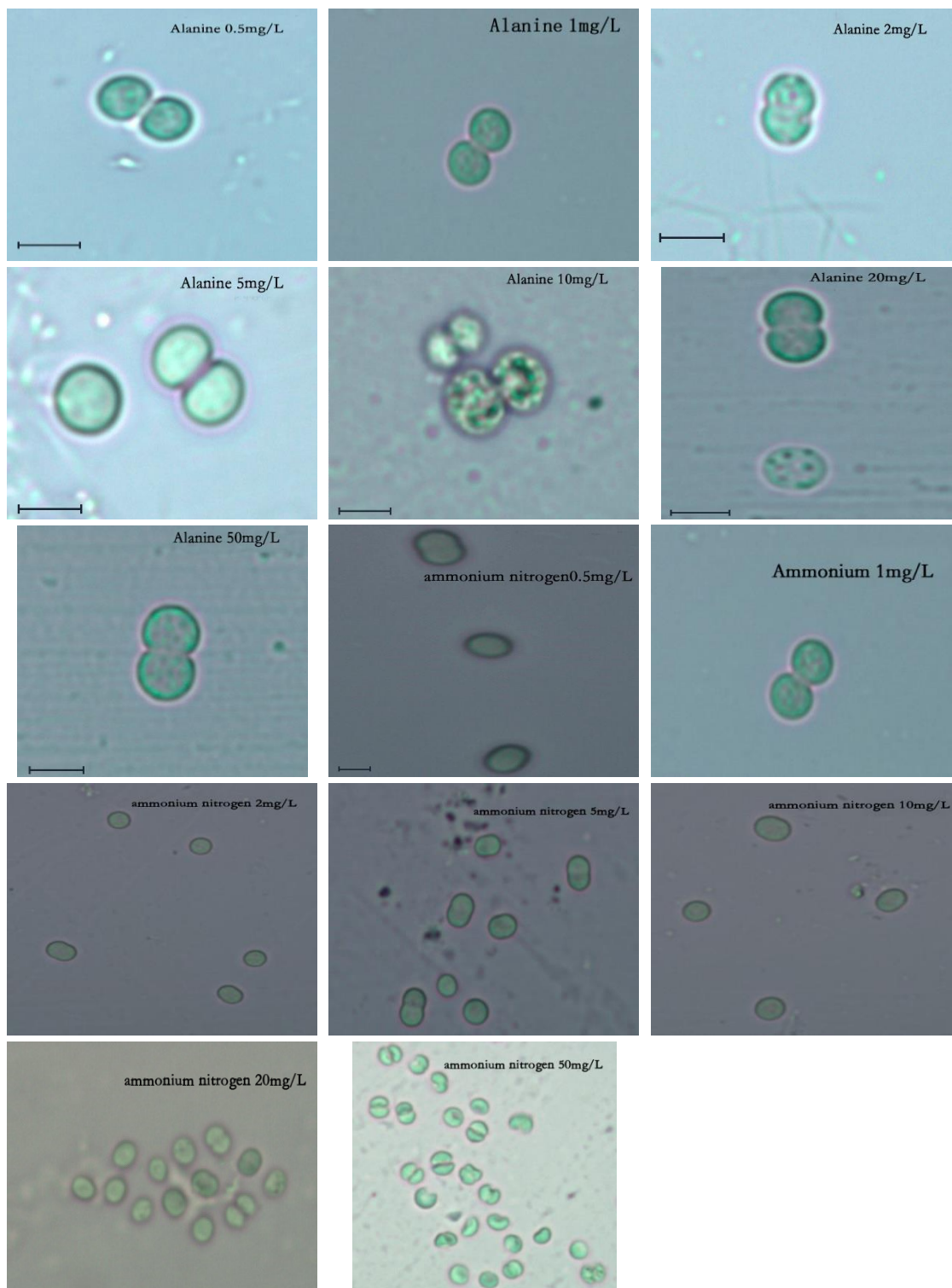
The plastid morphology of *S. quadricauda* under monoculture of two nitrogen sources is shown in Figure 7. In the ammonium treatment, *S. quadricauda* is mainly composed of two cells and four cells, and these cells were suffered seriously with the higher concentration of ammonium nitrogen. In the alanine experimental group, the content of chlorophyll of *S. quadricauda* was higher and the complete the plastid morphology was cell integrity with the increasing concentration of alanine, which indicated alanine benefited to maintain plastid integrity of *S. quadricauda*.



**Figure 7.** Cells of *S. quadricauda*. Scale bars indicate 10 μm

The plastid morphology of *M. aeruginosa* cultured with two nitrogen sources is shown in *Figure 8*. In the ammonium treatment group, the plastid of *M. aeruginosa* kept intact under the concentration of 0.5-20 mg/L  $\text{NH}_4\text{Cl}$ . When *M. aeruginosa* was cultured in 50 mg/L concentration of ammonium nitrogen, the cell chlorophyll showed a certain

reduction. In the alanine group, the *M. aeruginosa* cells in the 0.5-5 mg/L concentration treatment group remained intact. When the alanine concentration was more than 10 mg/L, the damage of *M. aeruginosa* cells was more and more serious. It can be seen from the figure that the plastid of *M. aeruginosa* can tolerate a high concentration of inorganic nitrogen, while it suffered more serious in high concentration of organic nitrogen.



**Figure 8.** Cells of *M. aeruginosa*. Scale bars indicate 10  $\mu\text{m}$

## Discussion

### *Effect of different nitrogen on the growth of M. aeruginosa and S. quadricauda*

From *Figure 1* and *Figure 2*, *M. aeruginosa* have a strong advantage in the utilization of ammonium, while *S. quadricauda* has a strong advantage in the utilization of alanine in the co-culture experiment. Because *M. aeruginosa* is a unicellular algae, it is generally believed that the utilization ability of unicellular algae to reduced ammonium salt ( $\text{NH}_4\text{Cl}$ ) is better than other nitrogen forms (Muro-Pastor and Florencio, 2003). This is mainly due to other nitrogen sources needing to be reduced to ammonia by nitrate reductase and nitrite reductase before these nutrients can be used. This reduction pressure forces *M. aeruginosa* to face the energy consumption for photosynthesis (Michard et al., 1996; Giani and Delgado, 1998; Meng et al., 2015). It is generally believed that alanine, as a small molecule organic compound, can not only enter cells through passive diffusion, but also enter cells through active transport. Moreover, *M. aeruginosa*, in the alanine treatment group, decreased with culture period (see *Figure 3*), which shows that not all phytoplankton can use organic nitrogen sources efficiently. Since *M. aeruginosa* effectively prefers to utilize ammonium nitrogen, we should pay attention to the role of microorganisms in promoting *M. aeruginosa* to become the dominant species through ammonification in lake. At present, the main management measure of eutrophic water body is to reduce the input of total nitrogen. However, Wu et al. (2013) pointed out that microbial community structure is very important for the change of available nitrogen sources into different forms. So algae control should not only be limited to the control of total nitrogen, but also focus on reducing the input of various nitrogen sources due to the ammonification of microorganisms.

Different algae species have different abilities to utilize different forms of nitrogen. Compared with *M. aeruginosa*, *S. quadricauda*, as multicellular algae, needs more nitrogen to maintain cell proliferation. Each cell of *S. quadricauda* has a peripheral and protein nucleus (Hu and Wei, 2006), and light energy is converted into chemical energy depending on the photosynthetic pigment in these melanosomes (Yan et al., 2012; Chandler et al., 2014). In this study, with the increase of  $\text{NH}_4^+$ -N concentration (see *Figure 7*), the degree of damage on melanosomes in *S. quadricauda* increased gradually, which directly affected its photosynthetic efficiency. However, when alanine was used as the nitrogen source, melanosomes of *S. quadricauda* were in good condition as the alanine concentration increased. Therefore, whether cultured alone or together with *M. aeruginosa*, the growth potential of *S. quadricauda* is better with alanine nitrogen than that of ammonium nitrogen, and it has better utilization advantages with alanine nitrogen than that of ammonium nitrogen. Hou et al. (2018) studied the relationship between phytoplankton succession and nutrients in Dianchi Lake and found that *S. quadricauda* was the main dominant species in the north of Dianchi Lake (Caohai Bay) with high total nitrogen and phosphorus in Caohai Bay, and *M. aeruginosa* is the dominant species in the south of Dianchi Lake (Waihai lake). This phenomenon is because that the Caohai Bay mainly receives the treated municipal wastewater with the higher organic matter while Waihai lake receives its main nitrogen source from agricultural non-point source pollution. Therefore, in the same period, *S. quadricauda* has a strong advantage in the utilization of organic nitrogen and developed into the dominant species in Caohai Bay, while *M. aeruginosa* is the dominant species in Waihai lake. In this study, it was found that in both ammonium and alanine nitrogen sources, *S. quadricauda* forms four-cell morphological algae structures that are sensitive to settle in water. This is conducive to

the growth of submerged plants and their strong competition for light and nutrition (Wang et al., 2009; Dong et al., 2013; Yang et al., 2015).

Different algae species not only have a distinguishable ability to use a variety of forms of nitrogen, but also have different requirements for nitrogen concentration for its growth. In our study, high concentrations of ammonium nitrogen ( $> 20$  mg/L) in the 16 days' culture period were beneficial to *M. aeruginosa* by increasing chlorophyll *a* content. This result did not coincide with reports which state high concentrations of  $\text{NH}_4^+$ -N can produce toxic effects on *M. aeruginosa* and affect the normal algae growth (Azov and Goldman, 1982; Zhou et al., 2013). *M. aeruginosa*'s chlorophyll *a* in the stress of high concentrations of ammonium chloride is to lag, so that the toxic effect of high concentrations of ammonium chloride is not obvious in the 16 days' culture cycle. However, other studies suggested that the growth of *M. aeruginosa* is limited when the ammonium nitrogen concentration reaches 50 mg/L (Dai et al., 2017). As autotrophs for photosynthesis, the photosynthetic activity of *M. aeruginosa* is directly related to the growth potential. When the ammonium nitrogen concentration is high ( $> 10$  mg/L), the photosynthetic activity Fv/Fm of *M. aeruginosa* is constantly rising, and higher than that of the low concentration of ammonium treatment group (see *Figure 4*). In terms of cell morphology of *M. aeruginosa*, when the ammonium nitrogen concentration is 0.5-20 mg/L, plastids remain intact. When the ammonium nitrogen concentration reaches 50 mg/L, the chlorophyll in the plastids of *M. aeruginosa* show slight damage. These results showed that *M. aeruginosa* prefers high concentrations of ammonium nitrogen because it can maintain high photosynthetic activity with sound plastid structure in ammonium nitrogen water. Some other research shows that there is no significant difference in the absorption of urea (organic nitrogen) and ammonium chloride by *M. aeruginosa*, and the absorption of these nitrogen forms by *M. aeruginosa* was significantly higher than that of nitrate-nitrogen (Xu et al., 2019). However, our study found that the chlorophyll *a* of *M. aeruginosa* in the high concentration of alanine nitrogen ( $> 20$  mg/L, organic nitrogen) was lower than that of other concentrations of alanine, and declined sharply as the culture time passed (see *Figure 3*). This indicated that not all small organic molecular forms of nitrogen can effectively promote *M. aeruginosa* growth. Alanine ( $\text{CH}_3\text{CH}(\text{NH}_2)\text{COOH}$ ) is a carboxyl compound and an acidic amino acid, and the pH value of the alanine culture medium is unfavorable to the pH environment required for the growth of *M. aeruginosa*. In the growth process, *M. aeruginosa* absorbs  $\text{CO}_2$  and  $\text{HCO}_3^-$ , through photosynthesis, and removes  $\text{H}^+$  in the culture medium increasing the pH value. The high pH environment rapidly accelerates the growth of *M. aeruginosa* (Yu et al., 2016; Zhu et al., 2018).

### ***Effects of different nitrogen on Photosynthetic Physiological Characteristics of M. aeruginosa and S. quadricauda***

One of the fundamental indexes reflecting the potential maximum photosynthetic capacity (photosynthetic efficiency) is the maximum conversion efficiency (Fv/Fm) of PS II which is the ratio of the maximum variable fluorescence to the total fluorescence. The measurement of Fv/Fm is often applied as a conventional means to investigate the response of algae to the environment because this parameter will decrease if algae is stressed by alien species or detrimental growth conditions (Björkman and Demmig, 1987; Krause, 1988; Xu et al., 1992; Han et al., 2005; Liu et al., 2019). The growth potential of algae in fresh water increases as photosynthetic activity increases, so the possibility of algae becoming the dominant species rises. In this experiment, the photosynthetic activity

(Fv/Fm) of *M. aeruginosa* was enhanced as the level of ammonium concentration increased, and the chlorophyll *a* increased correspondingly, which indicated that there was a certain positive correlation between the photosynthetic activity and biomass. With an increase of ammonium concentration, *M. aeruginosa* had higher photosynthetic activity and grew rapidly. *M. aeruginosa* had low Fv/Fm in 0.5 mg/L and 1 mg/L ammonium culture medium, and its growth potential was weakened, which may be the result of insufficient nutrients (Young and Beardall, 2003). Although chlorophyll *a* content of *M. aeruginosa* declined sharply with of the higher alanine concentration, the Fv/Fm of *M. aeruginosa* went up to the high value in the high alanine concentration (> 20 mg/L) culture medium, which may be because the high concentration of alanine did not cause any stress on the light energy conversion rate of *M. aeruginosa*, and it was not necessary to change the parameter Fv/Fm of photosynthetic activity to adapt to the high concentration of alanine environment (Wang, 2018). For *S. quadricauda*, the Fv/Fm in the high concentration ammonium culture medium (> 20 mg/L) was lower than that of the same concentration alanine treatment group, which suggested that the photochemical activity of PSII in *S. quadricauda* may be destroyed, and the photosynthetic electron transfer may be blocked under the stress of high concentrations of ammonium nitrogen. It is generally believed that Fv/Fm will be significantly reduced under the stress of high ammonium nitrogen or low ammonium nitrogen, which will lead to the decrease of biomass and chlorophyll fluorescence parameters (Young and Beardall, 2003). Most of the plastid of *S. quadricauda* are absent due to high concentrations of non-protonation ammonium nitrogen inhibiting photosynthetic activity (Dai et al., 2017). In this study, the plastid structure of *S. quadricauda* was damaged in the ammonium nitrogen culture medium, and the photosynthetic pigments in cells that convert light energy into stable chemical energy were also damaged hindering the process of photosynthetic electron transfer (Yang et al., 2015). It is a physiological reaction process for *S. quadricauda* to adapt to the stress of nutrients through the down regulation of photosynthesis (Lin et al., 1997). However, in the alanine nitrogen culture medium, Fv/Fm of *S. quadricauda* increased with the higher alanine concentration. As a small molecular amino acid, alanine can be absorbed into the cell by the way of active transport. The protein on the cell wall functions as a carrier, accelerating the rate of alanine entering the cell (Fang et al., 2013). Therefore, the growth potential of *S. quadricauda* in organic nitrogen water body is stronger than inorganic nitrogen water.

The higher the maximum photosynthetic rate (ETR<sub>max</sub>) indicates that algae are less susceptible to photo inhibition under strong light conditions, and the smaller the ETR<sub>max</sub> value indicates that the electron transfer is limited. The saturated light intensity (I<sub>k</sub>) is adaptable in algae to compensate strong light stress. Whether *M. aeruginosa* was cultured in alanine alone or in ammonium alone, *M. aeruginosa* had a higher maximum photosynthetic rate (ETR<sub>max</sub>) and a higher I<sub>k</sub> as the nitrogen source concentration increased in each culture. This showed that these photosynthetic parameters of *M. aeruginosa* were not sensitive to different nitrogen sources. The actual photosynthetic capacity of *M. aeruginosa* was not affected by the variety of nitrogen sources, and this undoubtedly increased the ecological range of *M. aeruginosa* to adapt to nitrogen, and also improved its survival probability and competitiveness. Some scholars suggested that temperature and light intensity were the main factors affecting the photosynthetic activity of algae rather than the chemical form of nutrients, finding that the ETR<sub>max</sub> value of some algae decreased significantly under 12800 lx light intensity (Zang et al., 2015). The change of Fv/Fm, ETR<sub>max</sub> and I<sub>k</sub> values in both ammonium and alanine nitrogen groups

was similar to that of chlorophyll *a* in *S. quadricauda*, indicating that there was no need for *S. quadricauda* to increase these parameters to adapt to the changes in ammonium nitrogen and alanine concentration gradients, while the other view was that under the stress of high ammonium nitrogen or nitrogen limitation, some chlorophyll fluorescence parameters of algae would increase to adapt to the changing living environment (Wang et al., 2012), the mechanism needs to be further explored.

## Conclusion

*M. aeruginosa* and *S. quadricauda* have strong advantage in the utilization of ammonium and alanine, respectively. The chlorophyll *a* and the photosynthetic activity Fv/Fm of *M. aeruginosa* is constantly rising in high concentrations of ammonium nitrogen (> 20 mg/L). Whether *M. aeruginosa* was cultured in alanine alone or in ammonium alone, *M. aeruginosa* had a higher maximum photosynthetic rate (ETR<sub>max</sub>) and the higher saturated light intensity (I<sub>k</sub>), indicating that these parameters of *M. aeruginosa* were not sensitive to different nitrogen sources and that this undoubtedly increased the ecological range of *M. aeruginosa* to adapt to nitrogen, and also improved its survival probability and competitiveness. For *S. quadricauda*, the plastid structure was damaged in the ammonium nitrogen culture medium, and the photosynthetic pigments in cells that convert light energy into stable chemical energy were also damaged hindering the process of photosynthetic electron transfer. Then the Fv/Fm of *S. quadricauda* was lower than that of the same concentration alanine treatment group.

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