The physiological responses of one algae species *Scenedesmus quadricauda* to ammonium and alanine

X L Hou¹, D L Ma¹, K F Yang¹, R Wang¹, D L Rong¹ and X T Yin¹,²

¹College of Agronomy and Life Sciences, Kunming University, Kunming 650214, China

E-mail: hxlyn@aliyun.com

Abstract. In order to explore the role of inorganic and organic nitrogen on physiological process of *Scenedesmus quadricauda*, we studied the influence of two nitrogen compounds, ammonium and alanine nitrogen on growth, physiological characteristics. Chlorophyll a and maximal photochemical quantum yield of PSII (Fv/Fm), maximum electron transport rate (ETRmax) and Saturating Light intensity (Ik) were measured by Phyto-PAM under the concentration of nitrogen 0.5, 1.0, 2.0, 5.0, 10, 20, 50 mg/L. Results indicated that Chlorophyll a of *Scenedesmus quadricauda* was the lowest in 0.5 mg/L and 50 mg/L, and it was the highest value in 5 mg/L and 10 mg/L in ammonium nitrogen treated group. In alanine experiment group, Chlorophyll a of *Scenedesmus quadricauda* was raised with the increase of alanine concentration and was positive relation with alanine concentration. These results showed that ammonium (<5 mg/L and >20 mg/L) inhibited the growth of *Scenedesmus quadricauda* and that alanine nitrogen is more easily for *Scenedesmus quadricauda* to take up. Fv/Fm, ETRmax and Ik of *Scenedesmus quadricauda* showed different tendency between ammonium and alanine experiment group. These parameters showed higher in 5 mg/L and 10 mg/L than that of the other concentration in ammonium experiment group. In alanine experiment group, Fv/Fm, ETRmax and Ik of *Scenedesmus quadricauda* were rising with higher alanine concentration and the fluctuation of these values of these parameters was in a small range. *Scenedesmus quadricauda* was sensitive to low ammonium concentration (<5 mg/L) and high ammonium concentration (>20 mg/L). The higher alanine concentration promoted the growth of *Scenedesmus quadricauda*.

1. Introduction

In water bodies, nitrogen (N) have all kinds of forms, for example, these dissolved nitrogen include inorganic nitrogen, nitrate (NO$_3$--N), nitrite (NO$_2$--N), ammonium nitrogen (NH$_4$+--N), and organic nitrogen, amino acid, carbamide, amide, vitamin and so on. In general, algae directly tend to take up nitrate (NO$_3$--N) and ammonium nitrogen (NH$_4$+--N) [1], ammonium nitrogen (NH$_4$+--N) promoted the growth of cyanobacteria and make these cyanobacteria dominant species, and then it is possible to erupt cyanobacterial blooms. But in high content of nitrate (NO$_3$--N) water body, green algae is easy to be dominant species and green algae don’t form algae blooms and can restrain the growth of cyanobacteria in some extend [2]. *Scenedesmus quadricauda* restrain the photosynthetic activity of *Microcystis aeruginosa*, and then affect the growth of cell of *Microcystis aeruginosa* and make growth rate of the biomass decline [3]. At present, most of study focuses on mechanism of cyanobacterial production toxin, and effects of physical factors such as total nitrogen and phosphorus ratio of N/P, highlight, temperature on physiological indicators of cyanobacteria [4,5]. But the physiological
characteristic of green algae during its growth is not paid enough attention, so it is difficult to explore the inhibition mechanism of *S. quadricauda* on cyanobacteria. Wan et al. studied on the growth of *S. quadricauda* under different nutritional conditions and found that competitive inhibition of *S. quadricauda* on *Microcystis aeruginosa* at eutrophic level is related to N/P ratio [6]. The water body of Dianchi Lake in Yunnan is seriously eutrophicated, and the water quality is inferior to category V. In 2001-2009, the dominant species are aphanizomenon flos-aquae and *S. quadricauda* of chlorophyta and *Microcystis aeruginosa* of cyanophyta in the community of phytoplankton and these phytoplankton alternates with each other in different seasons [7]. The total nitrogen and phosphors are higher in the northern water body than that of southern water body of Dianchi Lake, and *S. quadricauda* is dominant species in most seasons and its’ biomass are higher than *Microcystis aeruginosa* [4]. This is not consisting with that Microcystis without nitrogen fixation ability become dominant species in high nitrogen and phosphorus water body [8,9]. Since *S. quadricauda* become dominant algae species in Dianchi Lake with high N/P and high N/P ratio, does it have a certain advantage in utilizing certain forms of nutrients or not? This aspect still needs to be further explored.

As an autotrophic organism, algae photosynthesis has an effect on the physiological process and various structures of algae, and closely associated with the growth of algae [10]. To indentify effects of nutrients in different occurrence forms on *S. quadricauda*, we carried out experiments on the growth of *S. quadricauda* in organic and inorganic nitrogen sources and study on photosynthesis and cell morphology of *S. quadricauda*.

2. Methods

2.1. Algae treatment and experimental design

*S. quadricauda* was obtained from the Freshwater Algae Culture Collection of the institute of Hydrobiology (FACHB-collection, Wuhan, China). Prior to experiments, *S. quadricauda* cells were harvested in the logarithmic growth period by centrifugation (4000 rpm, 8 min), rinsed 3 times with N- and P-free BG11 medium and then inoculated in medium for 48 h to exhaust nutrients stored in cell. At the onset of the experiment, monocultures of *S. quadricauda* were exposed to two nitrogen sources (Alanine and ammonium nitrogen) in the experiment. Iron citrate replaced ammonium ferric citrate and cobalt chloride replaced with cobalt nitrate, in order to eliminate effect of nitrogen on *S. quadricauda*. The ratio of other components in BG11 medium was the same. In two experiments, The different nutrient treatments were obtained by modifying the alanine and ammonium nitrogen concentrations in regular BG11 medium. Concentration gradients of ammonium nitrogen and alanine were 0.5, 1.0, 2.0, 5.0, 10, 20, 50 mg/L in two experiments and each treatment group is triplicate.

2.2. Measurement of physiological parameters and data processing

The parameters of chlorophyll a, photosynthetic activity and fast light response curve were measured every two days using by Phyto-PAM (WALZ, Germany). The photochemical efficiency of photosystem II (Fv/Fm) was determined according to Ting and Owens (1992) and the values of maximum relative electron transport rate (ETRmax) were plotted vs irradiance to give the corresponding rapid light curve, which was automatically recorded by preset software in the Phyto-PAM. 25. The measured parameters included chlorophyll a content, photosynthetic activity (Fv/Fm) and maximum electron transfer rate (ETRmax) and Saturating Light intensity (Iₛ). Statistical analyses and figures for environmental data were completed using Microsoft Excel (2010) and origin 8.0.

3. Result

The variation of chlorophyll a content in *S. quadricauda* is shown in figure 1. At the end of the experiment, the chlorophyll a content of *S. quadricauda* was 2.87 mg/L in two treatment of 0.5 mg/L and 50 mg/L ammonium nitrogen concentration and these are the lowest value in all ammonium treatment groups, and that content of the chlorophyll a of these two treatment was lower than that of
other treatment group in whole growth period. The chlorophyll a content of *S. quadricauda* in two treatments of 5 mg/L and 10 mg/L ammonium nitrogen concentration are the highest in all these ammonium nitrogen treatments when *S. quadricauda* grewed up until the end of experiment, and that the chlorophyll a content of *S. quadricauda* in 5 mg/L ammonium nitrogen treatments is higher than that of the other treatments in whole growth period. These results indicated that the lowest concentration of 0.5 mg/L and the highest concentration of 50 mg/L ammonium nitrogen water body was not conducive to the growth of *S. quadricauda*.

![Figure 1. Content of chlorophyll a of *S. quadricauda*.](image)

The change of chlorophyll a content of *S. quadricauda* in the treatment of alanine was significantly different from that in the treatment of ammonium nitrogen. In general, the chlorophyll a content of *S. quadricauda* of 50 mg/L and 20 mg/L alanine nitrogen concentration treatments was higher than that of the other treatments and the values were 7.30 mg/L and 5.89 mg/L respectively. The chlorophyll a content of *S. quadricauda* of 1 mg/L and 5 mg/L alanine nitrogen concentration treatments was lower with the value of 3.82 mg/L, 3.47 mg/L respectively. In the process of growth of *S. quadricauda*, the chlorophyll a content in 50 mg/L alanine nitrogen concentration treatments was the highest among these concentration treatments. The growth of *S. quadricauda* at the concentration of 20 mg/L alanine was the second.

Comparing the growth trends of *S. quadricauda* in ammonium nitrogen and alanine treatment experiment, we found that the growth of *S. quadricauda* was the best when the concentration of ammonium nitrogen was 5 mg/L and 10 mg/L, and its chlorophyll a content increased nearly three times as much as the initial period and the growth of *S. quadricauda* tends to slow with the increase of ammonium nitrogen concentration. At all alanine concentrations treatments, the growth of *S. quadricauda* in 1 mg/L and 5 mg/L alanine concentration was the worst, even so, chlorophyll-a content of these treatment increased nearly three times as much as in the initial period. The content of chlorophyll-a of *S. quadricauda* treated with alanine at 50 ml/L increased rapidly and reached 7.3 mg/L. Organic nitrogen (alanine) is more beneficial to the growth of *S. quadricauda* than inorganic nitrogen (ammonium).

Photosynthetic activity of *S. quadricauda* was significantly different between ammonium nitrogen and alanine treatments (figure 2). In different ammonium concentration treatments, the average of photosynthetic activity (Fv/Fm) of *S. quadricauda* in 5 mg/L and 10 mg/L ammonium concentration treatments is the highest with the value of 0.74 and 0.75, respectively, and it’s range of photosynthetic activity are 0.68–0.80and 0.66–0.87, respectively during the growth process. The average of photosynthetic activity (Fv/Fm) of *S. quadricauda* in 0.5 mg/L and 1 mg/L ammonium concentration treatments are the lowest among all concentration ammonium treatments, and the value of Fv/Fm grow rapidly with the increasing ammonium concentration (0.5 mg/L to 10mg/L ammonium concentration), and the maximum photosynthetic activity was achieved in treatment with 5 mg/L and
10 mg/L ammonium nitrogen, and then the value of photosynthetic activity decreased gradually with the increasing ammonium concentration. These results showed that *S. quadricauda* in low (<0.5 mg/L) and high (>20 mg/L) concentrations of ammonium nitrogen have very low photosynthetic activity, and the photosynthetic activity of *S. quadricauda* in medium concentration of ammonium nitrogen was stronger.

![Figure 2. Photosynthetic activity of *S. quadricauda*.](image)

In 0.5 mg/L, 1 mg/L, 5 mg/L, 10 mg/L, 20 mg/L, 50 mg/L alanine concentration treatments, *S. quadricauda* has the highest average photosynthetic activity (Fv/Fm) in 20 mg/L and 50 mg/L concentration of alanine experiments with the same value 0.75. In the growth period, the range of Fv/Fm in 20 mg/L and 50 mg/L alanine experiments is 0.66~0.87 and 0.67~0.81, respectively. The Fv/Fm of *S. quadricauda* increased with increasing concentration alanine, and these results showed high concentration of alanine treatment of *S. quadricauda* has strong growth potential.

![Figure 3. Maximum photosynthetic rate of *S. quadricauda*.](image)

Maximum electron transport rate (ETRmax) is characterization of photosynthesis efficiency of phytoplankton. The ETRmax of *S. quadricauda* treated with ammonium nitrogen and alanine is shown in figure 3. Among ammonium treatments, ETRmax of *S. quadricauda* declined with the time of cultivation except 10 mg/L ammonium nitrogen treatment. ETRmax of *S. quadricauda* treated with 10 mg/L and 5 mg/L ammonium nitrogen treatment is higher than that of other treatments in grow period, and the lowest ETRmax occurred in 0.5 mg/L and 1mg/L ammonium nitrogen treatments. In all
alanine treatments, ETRmax showed is as follows, that the ETRmax of *S. quadricauda* in 50 mg/L alanine treatment group was higher than that of other treatment groups in the growth cycle. The lowest ETRmax value was found in low concentration alanine treatment group (0.5 mg/L and 1 mg/L). The ETRmax of *S. quadricauda* treated with alanine gradually decreased with culture time.

Saturated light intensity ($I_k$) represents the adaptability of phytoplankton to light intensity. The result (in figure 4) showed that the median value of $I_k$ in 0.5 mg/L ammonium nitrogen treatment group was the lowest, and that the median value of $I_k$ in 20 mg/L and 50 mg/L ammonium nitrogen treatment group was second. The median value of $I_k$ in 5 mg/L and 10 mg/L ammonium nitrogen treatments was higher than that in other treatments and had a small fluctuation range. These result indicated that the ability of high light tolerance of *S. quadricauda* treated with medium concentration of ammonium nitrogen was higher than that of other groups. The saturated light intensity ($I_k$) of alanine treatment group showed the following regularity, which the median value of saturated light intensity ($I_k$) of *S. quadricauda* increased with the increase of alanine concentration. The highest $I_k$ of *S. quadricauda* occurred in 50 mg/L alanine concentration treatments and the fluctuation range of this value is small, indicating that the ability of *S. quadricauda* to withstand strong light in high concentration alanine treatment group was stronger than that of low concentration group.

![Figure 4. Saturating Light intensity Scenedesmus quadricauda.](image)

The cell morphology is shown in the following figure 5. In the ammonium nitrogen treatment group, two and four cells of *S. quadricauda* were dominant. In 0.5~2 mg/L ammonium nitrogen treatments, cell damage is more serious. When the concentration of ammonium nitrogen increased, the cells of *S. quadricauda* remained intact. In the alanine nitrogen treatment group, the higher the concentration of alanine was, the higher the chlorophyll content was. That is to say, Cell morphology showed that alanine is beneficial to the growth of *S. quadricauda*. 

![Image of cell morphology](image)
Figure 5. Damage degree of plastid of *S. quadricauda* in the experiment.

4. Discussion and conclusion

There are various forms of nitrogen sources in lake water for phytoplankton photosynthesis. When the form of nitrogen source changes, the physiological process of algae will be affected, and then effects on chlorophyll a content directly or indirectly. Therefore, the change of chlorophyll a content can be regarded as a physiological response index of algae under environmental stress [11]. Ammonia nitrogen is an available nitrogen source for algae, but in this study these values of chlorophyll a content, photosynthetic activity, photosynthetic efficiency and adaptability to strong light of *S. quadricauda* were the highest at 5 mg/L and 10 mg/L ammonium nitrogen concentration treatment. This indicates that there is a certain threshold range of ammonium nitrogen suitable for the growth of
S. quadricauda. The effects of different concentrations of ammonium nitrogen on chlorophyll a content of S. quadricauda were different. At the later stage of the experiment, the chlorophyll a content of S. quadricauda in 0.5 mg/L and 50 mg/L ammonium nitrogen treatments was the lowest and the difference between two groups was not obvious, these result showed that the response of S. quadricauda to low and high concentration of ammonium nitrogen is sensitive. High concentration of ammonium nitrogen increases the osmotic pressure inside and outside the cell of S. quadricauda, thus affects the transport and absorption of substances, and then resulted in different inhibition phenomena [12]. Low concentration of ammonium nitrogen shows a nitrogen-limited state for S. quadricauda growth, which may lead to the conversion of excess carbon and hydrogen to the synthesis of non-nitrogen pigments in the cells of S. quadricauda [13]. From the cell morphology in this experiment, low concentration ammonium nitrogen cells were seriously damaged (see figure 5). Of course, the mechanism still needs to be further explored.

Alanine of different concentration has a good promoting effect on the growth and reproduction of S. quadricauda. Alanine with the same concentration of ammonium nitrogen promoted the growth of S. quadricauda more than ammonium nitrogen. And the growth of S. quadricauda increased with the increase of alanine concentration. These result indicated that the promotion of small molecular amino acids such as alanine on the growth of S. quadricauda was obvious. The 5 mg/L and 10 mg/L ammonium nitrogen concentration obviously promote the growth of S. quadricauda, but chlorophyll a content of S. quadricauda was close to that of 5 mg/L in the alanine experiment on the 16th day and was far less than that of 50 mg/L the alanine concentration treatments. The chlorophyll a content of S. quadricauda increased with the increase of alanine concentration. Except nitrogen concentration in water has a certain effect on the growth and reproduction of S. quadricauda, nitrogen forms plays an important role in the growth and reproduction of S. quadricauda. This was confirmed by previous reports [14].

Photosynthetic activity (Fv/Fm) is affected by illumination, nutrient concentration and phytoplankton structure [15], and it is an important indicator of the physiological characteristics of phytoplankton. In ammonium nitrogen concentration treatments, the Fv/Fm values show a decreasing trend, when the concentration of ammonium nitrogen is more than 10 mg/L or less than 5 mg/L. Wang et al. research that the Fv/Fm of A. pseudoanabaena decreased with the increase of culture days and the decrease of nitrogen concentration, and the Fv/Fm was positively correlated with nitrogen concentration [16]. Our study showed that S. quadricauda has high growth potential and strong competitive advantage in the concentration range of 5-10 mg/L ammonium nitrogen. In general, the Fv/Fm decreased significantly when algae were stressed by high concentration of ammonium nitrogen or limited by low concentration of ammonium nitrogen. Our results are consistent with the results of most studies that too high or too low nutrient concentration will lead to the decrease of biomass and chlorophyll fluorescence parameters [17]. High and low concentration of ammonium nitrogen inhibited the photosynthetic activity of S. quadricauda because high concentration of non-protonated ammonium nitrogen resulted in the loss of most of the pigments in S. quadricauda [18], and then hindered the process of photosynthetic electron transfer in S. quadricauda [19]. The obstruction of photosynthetic electron transfer process will affect the utilization efficiency of light energy and the potential maximum photosynthetic rate, which is reflected that the change trend of ETRmax and I הם values in ammonium nitrogen experimental group is the same as that of Fv/Fm (see figures 3 and 4).

In alanine treatments, the Fv/Fm of S. quadricauda increased with the increase of alanine concentration. This is mainly because alanine, as a small molecule amino acid, can be absorbed into algae cells through active transport and at this time, proteins on the cell wall act as carriers and accelerate the rate of alanine entering algae cells [20]. So the growth potential of S. quadricauda was stronger than that of inorganic nitrogen when high concentration of organic nitrogen was used as nitrogen source. In our study, It was found that the trends of Fv/Fm, ETRmax and Ición values in ammonium nitrogen group and alanine group were similar to those of chlorophyll a content, indicating that the gradient of ammonium nitrogen and alanine concentration did not need to increase chlorophyll fluorescence to adapt to different nitrogen sources. But chlorophyll fluorescence parameters of D.
aureus increased to adapt to the living environment under ammonium nitrogen stress or nitrogen restriction [21]. The mechanism of S. quadricauda adapted to ammonium nitrogen still needs to be further explored.

Acknowledgments
This work was supported by special project of Basic Research in Yunnan Local Colleges and Universities (2017FH001-026) and the Nation Natural Science Foundation of China (31300349).

References
[1] Zhang Q T, Wang X H, Lin C, Hu G K and Guo Y 2011 Effects of different nitrogen on proliferation of Microcystis aeruginosa J. Hydroecol. 32 115-20
[2] Zhu L 2007 Effects of different P concentration, nitrogen sources and aeration patterns on growth of algae in freshwaters (Chongqing, China: Chongqing University)
[3] Fang L, Liu W Q, Zhao N J, Duan J B and Wang Z G 2013 The influence of Scenedesmus quadricauda on MC-LR producing, releasing and photosynthetic activity of Microcystis aeruginosa J. Biol. 30 33-7
[4] Pang N Y, Dai G F, Zhang W, Ge G, Yang P, Guo C J and Fang Y Y 2018 Differences in nutrition condition and algae population in different areas of Poyang Lake Journal of Lake Sciences 1295-308
[5] Hou X L, Yuan C G, Li X P, Ren Y C, Luo Y M and Wang D K 2018 Effect of nitrogen and phosphorus concentrations on the planktonic algae dynamics in Dianchi Lake J. Hydroecol. 39 16-22
[6] Wan L, Zhu W and Zhao L F 2007 Effect of nitrogen and phosphorus on growth and competition of M. aeruginosa and S. quadricauda Environ. Sci 28 1230-5
[7] Guo L L, Zhou W C, Zhou Q C and Li G B 2015 Effects of light intensity and phosphorus concentration on the growth of Cladophora oligoclonia China Environ. Sci. 35 2153-9
[8] Wang L Y, Sang M, Li A F and Zhang C W 2012 Effects of different nitrogen nutrition level on the growth and photosynthetic physiology of odontella aurita China Biotechnology 32 48-56
[9] Li Y, Zhang M and Wang R N 2005 The temporal and spation variation of the cyanobacteria which caused the water bloom in the Dianchi Lake, Kunming, China J. Yunnan Univ. 27 272-6
[10] Lv X J, Zhu J and Meng L 2010 Pilot study on diversity of Cyanobacteria bloom in Erhai Lake Environ. Sci. Surv. 29 32-5
[11] Jia X H, Shi D J, Shi M H, Li R H, Song L R, Fang H, Yu G L, Li X and Du G S 2011 Formation of cyanobacterial blooms in Lake Chaohu and the photosynthesis of dominant species hypothesis Acta Ecologica Sinica 31 2968-77
[12] Tan X, Dai K W, Duan Z P, Li N G, Gu H H and Shu X Q 2018 Comparison of the effects of naphthalene on the growth and chlorophyll fluorescence of Microcystis aeruginosa and Synechococcus sp J. Hohai Univ. (Nat. Sci.) 46 115-21
[13] Lippemeier S, Klaush R H, Hartig V P and Colijn F 2001 In-line recording of PAM fluorescence of phytoplankton cultures as a new tool for studying effects of fluctuating nutrient supply on photo synthesis Eur. J. Physio. 36 89-100
[14] Wang Y J, Sun M Z, Li A F and Zang C W 2014 Effects of Nitrogen Concentration on the Growth and Photosynthetic Physiology of Scenedesmus acuminatus China Biotech. 34 51-8
[15] Wu X H, Gao J Y, Yan Y W, Zhou B and Dai R H 2015 Comparison of inorganic nitrogen and organic nitrogen on the growth and microcystin production of Microcystis aeruginosa Acta Scientiae Circumstantiae 35 677-83
[16] Wang M W 2018 Effect of environmental factors on growth and photosynthetic physiology of Pseudanabaena sp (Qingdao, China: Qingdao University of Technology) pp 36-9
[17] Young E and Beardall J 2003 Rapid ammonium and nitrate-induced perturbations to chl a fluorescence in nitrogen-stressed Dunaliella tertioleca (Chlorophyta) J. Phycol. 39 332-42
[18] Dai L L, Guo L L, Li L, Zhou W C and Li G B 2017 The responses of two algal species, *Microcystis aeruginosa* and *Scenedesmus quadricauda* to ammonium *Chin. J. Ecol.* **36** 2289-95

[19] Ting C S and Owens T G 1992 Limitations of the pulse-modulated technique for measuring the fluorescence characteristics of algae *Plant Physiol.* **100** 367-73

[20] Zhu G W, Qin B Q, Zhang Y L, Xu H, Zhu M Y, Yang H W, Li K Y, Min S, Shen R J and Zhong C N 2018 Variation and driving factors of nutrients and chlorophyll-a concentrations in northern region of Lake Taihu, China, 2005—2017 *J. Lake Sci.* **30** 279-95

[21] Sauer N, Komor E and Tanner W 1983 Regulation and characterization of two inducible amino-acid transport systems in Chlorella vulgaris *Planta* **159** 404-10