RESEARCH ARTICLE

Biotoxicity of NH_4^+ on *Nostoc Sphaeroides* (diazotrophic cyanobacteria) in paddy floodwater

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Abstract: In order to elucidate the toxicity of NH_4^+ on *Nostoc Sphaeroides* (commonly named GE-XIAN-MI in China, GXM), a diazotrophic cyanobacterium in paddy fields, this study investigated the growth, photosynthesis, N_2-fixation, and oxidative-antioxidative characteristics of GXM under NH_4^+ stress in paddy floodwater. The results showed that the photosynthesis and the N_2-fixing ability of GXM were inhibited by NH_4^+ at a concentration of 13.46 mg L^{-1} in paddy floodwater. The main target of NH_4^+ on photosystem II (PS II) of GXM was the electron donor side of react center. Oxygen evolution complex (OEC) was the secondary target of toxicity of NH_4^+ on PS II. The damage of PS II led to accumulation of ROS and resulted in oxidative damage on plasma membrane of GXM. Feedback inhibition and decrease of photosynthetic energy production may be the reasons for the decrease of N_2-fixing ability of GXM under NH_4^+ stress. The results presented in this study suggest that high level of NH_4^+ loading may be a reason for the recession of GXM resource in paddy fields.
Keywords: *Nostoc Sphaeroides*, ammonium, paddy floodwater, photosynthesis, nitrogenase, oxidative damage

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1 Introduction

Nitrogen (N) is the main nutrient element necessary for plant. The application of N chemical fertilizer is one of the main ways of N loading in agricultural soils (Shrestha et al., 2022). However, long-term of N chemical fertilizer application has also led to a series of environmental problems, such as soil acidification, eutrophication, and greenhouse gas emissions (Guo et al., 2010; Zhu et al., 2020). Diazotrophic cyanobacteria are important microbes in paddy ecosystems (Shao et al., 2021; Akoijam et al., 2015), and these microorganisms can reduce the usage of N chemical fertilizers in paddy fields through biological N_2-fixation (Jiang et al., 2022; Jiang et al., 2021). Previous study showed that the amount of N loading by diazotrophic cyanobacteria in paddy field reached 20-60 Kg N ha^{-1} per rice season. The addition of diazotrophic cyanobacterium fertilizer reduces 50% of N fertilizer in paddy fields (Abinandan et al., 2019). Besides N input, diazotrophic cyanobacteria can also load organic carbon into agricultural soil through photosynthesis (Gheda and Ahmed, 2015). Some diazotrophic cyanobacteria can promote growth of rice through synthesize and secret plant growth-promoting hormones (Sharma et al., 2020). And also, some diazotrophic cyanobacteria could hydrolyze organophosphorus and increase the level of bioavailable P in paddy field (Subramanian et al., 1994; Hu et al., 2022).

Nostoc Sphaeroides (GE-XIAN-MI, GXM,) is an edible diazotrophic cyanobacterium in rice fields. In addition to its high nutritive characteristics, GXM plays an important role in N input in paddy fields. Qin et al. (2013) found that paddy cultivation of GXM significantly increased the N content in the surface (0-5 cm) and subsurface (5-10 cm) layers of soil. Also, the decomposition of GXM biomass under flooding conditions is an important pathway for N input in paddy fields. Thus, GXM in rice fields has a great prospect in reduction of N chemical fertilizer application and promoting rice yield.

For N nutrient, rice prefers NH_4^+-N relative to NO_3^--N (Chen et al., 2022). However, previous studies showed that N chemical fertilizers, mainly ammonia, exhibited biological toxicity on GXM. Ammonia produced by the hydrolysis of NH_4^+ is a structural analogue of H_2O molecule. High concentration of NH_3 in cyanobacterial cells would bind to D1 protein in oxygen evolution complex (OEC) and interfere in the binding of OEC toward H_2O, thus affect the function of OEC (Drath et al., 2008; Evans et al., 2005; Dai et al., 2008). Dai et al. (2008) reported that the growth of GXM was inhibited by NH_4^+ at a concentration of 1 mmol L^{-1} in BG11 medium. Ammonium stress inhibits the photosynthesis of GXM (Dai et al., 2008). The bio-toxicity of NH_4^+ could be influenced by the ambient environments. Kater et al. (2006) found that the LC_{50} of NH_4^+ on *Corophium volutator* significantly decreased with increase of ambient pH. Considering that the composition and stability of pH between BG11

medium and paddy floodwater are significantly different, the toxicity of NH_4^+ on GXM in paddy floodwater may be different to that in BG11 medium. To fill this research gap, this study investigated the bio-toxicity of NH_4^+ on GXM in paddy floodwater through photosynthesis, oxidant-antioxidant characteristics and N_2-fixing rate. We hypothesized that: 1) the toxic threshold of NH_4^+ on GXM in paddy floodwater would be different to that in BG11 medium; 2) the damage of photosystem of GXM may induce oxidative stress and result in oxidative damage; 3) high level of NH_4^+ would affect N_2-fixing rate of GXM in paddy floodwater.

2 Materials and Methods

2.1 Cyanobacterial strain

GXM was provided by Dr. Huo Da (Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, China). Spherical GXM colony was surface sterilized using ethanol and homolyzed in a sterile mortar, and then cultured in BG11 medium at 25±1°C under shaking. The light intensity of the incubation chamber was 30 μmol photons m^{-2} s^{-1} with an illumination/darkness cycle of 12 h: 12 h.

2.2 Simulation of paddy floodwater

The paddy soil was collected from the base of Cultivation Garden of Hunan Agricultural University (28°18'25" N, 113°07'34" E). Simulation of paddy floodwater were prepared according to the methods described by Yan et al. (2022). In brief, deionized water (dH_2O) was mixed with soil in a ratio of 10:9 (*W*:*W*), with a flooding depth of 1cm, and incubated at 25°C under darkness for 15 d. Paddy floodwater was obtained by centrifugation at 12,000 $\times g$ for 10 min. The paddy floodwater was sterilized by filtration using 0.22 μm cellulose acetate membrane. The physicochemical properties of paddy floodwater were as follows: total N, 12.59 mg L^{-1}; NH_4^+-N, 0.322 mg L^{-1}; NO_3^--N, 6.76 mg L^{-1}; total P, 0.14 mg L^{-1}; bio-available P, 0.0617 mg L^{-1}; chemical oxygen demand, 692.3 mg L^{-1}.

2.3 Experimental design

The toxic experiments of NH_4^+ (NH_4Cl) on GXM were carried out in 250 mL conical flasks. Equal volumes of NH_4Cl solution with different concentrations were added into conical flasks containing filtration-sterilized paddy floodwater, and then aliquot volume of exponential phase GXM microspheres ($d < 0.1$ mm) was added into conical flasks to achieve an initial concentration of chlorophyll *a* (Chl *a*) 0.55 mg L^{-1}. The final volume of each treatment was 100 mL, and the final concentration of NH_4^+ was 0.41 (background concentration in control treatment), 13.46, 26.92, and 67.30 mg L^{-1}, respectively. In order to maintain a stable pH during incubation, citric acid - sodium citrate buffer (40 mM, pH 7.0) was added into conical flasks. A pre-experiment in our lab showed that citric acid - sodium citrate buffer at this concentration had no significant effect on growth and photosynthesis of GXM during the experimental period. Each treatment set 3 replicas. All treatments were incubated under the conditions described in section 2.1. Samples for physiological analyses were taken after 24 h of incubation. Due to low growth rate of GXM, the samples for growth analysis were sampled on the 96^{th} h. Growth rates were determined using the following formula, where C_{t1} and C_{t2} are the Chl a concentrations at time t_1 and t_2, respectively.

$$\text{Growth rate} = (\ln C_{t2} - \ln C_{t1})/(t_2 - t_1)$$

2.4 Determination of photosynthetic pigments and growth characteristics

The cells of GXM are compacting to form small spherical colony. Thus, it is difficult to determine cell population density using microscope. In this study, the growth characteristics of GXM was evaluated by the variation of Chl *a* concentration in conical flasks. Chl *a* in cells of GXM was extracted using 95% ethanol and determined spectrophotometrically at 665 nm, 649 nm (Yan et al., 2022). Chl a concentration was calculated using the following equations.

$$\text{Chl } a(\text{mg/L}) = 13.95\times A_{665} \times A_{649}$$

2.5 Enzymes extraction and phycobiliproteins content determination

The enzyme solution of GXM was extracted by liquid nitrogen grinding method. Concentration of total protein in enzyme solution was determined by Coomassie Brilliant Blue staining method. The contents of phycobiliproteins, viz. phycoerythrin (PE), phycocyanin (PC), and allophycocyanin (APC), were determined spectrophotometrically at 620, 650 and 565 nm, and then calculated according to the method described by Glazer (1984).

2.6 Measurement of chlorophyll fluorescence transients (CFT)

CFT of GXM was determined by a fluorometer (AquaPen AP-C 100, Photon Systems Instruments Co., Brno, Czech Republic) after 15 min of dark incubation (Lin et al., 2017). The parameters, PI_{abs}, φP_0, ψ_0, φE_0, $(1\text{-}V_k/V_j)_r$ and φD_0, originated from CFT were analyzed using the method described by Christen et al. (2007).

2.7 Measurement of catalase (CAT) activity

The activity of CAT was assayed by its ability in decomposing of H_2O_2. In brief, the crude enzyme solution or phosphate buffer (control) and H_2O_2 (0.0125 mol L^{-1}) were mixed and incubated at 25°C for 1 h, and then stopped by H_2SO_4 with a final concentration of 0.23 mol L^{-1}. The residual H_2O_2 in the reaction mixture was determined by an ammonium molybdate spectrophotometry method (Qing et al., 2020).

2.8 Determination of reactive oxygen species (ROS) and malondialdehyde (MDA) content

The content of ROS in algal cells was determined using a reduced dichlorofluorescein diethyl ester staining method (Hong et al., 2008). The content of MDA in algal cells was determined by a thiobarbituric acid method (Del et al., 2005).

2.9 Dinitrogen-fixing rate determination

The N_2-fixing rate of GXM was determined using the acetylene reduction method. Forty milliliters GXM cultures were sampled and centrifuged at 8000 × g for 5 min at 4°C, and after removal of 35 mL supernatant, the remaining sample was transferred into a 120 mL serum vial. Twelve milliliters of air was extracted by a syringe, and then an equal volume of acetylene was injected into it. After 24 h of incubation under light, the content of acetylene reduction product, viz. ethylene, was detected on a gas chromatography (Agilent GC-7890) equipped with a CP-Al_2O_3/KCl capillary column (50 m × 0.53 mm × 10 μm) and hydrogen flame ionization detector (FID). The temperature program of chromatogram was as follows: 35°C for 0.5 min, then increased to 70°C with a speed of 8°C min^{-1}, and then maintained at 70°C for 1 min. The temperature of injection and FID detector was 120°C and 300°C, respectively. High purity N_2 was used as carrier gas.

2.10 Statistic analyses

Data from this study were analyzed by One-Way ANOVA (LSD) using SPSS 13.1. Difference was regarded as significant at $p < 0.05$.

3 Results

3.1 Growth characteristics

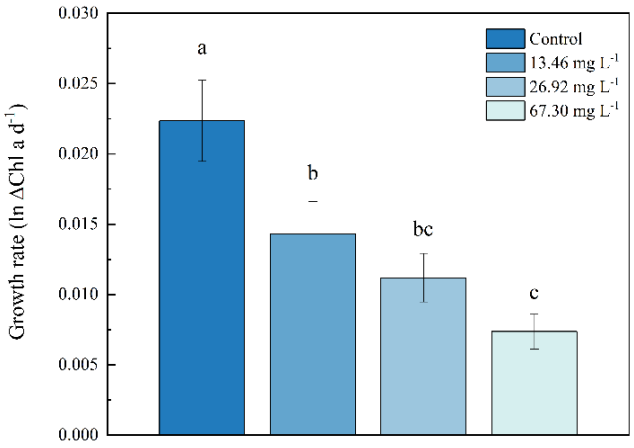

Figure 1. Effects of NH_4^+ on the growth rate of GXM (*N. Sphaeroides*) in paddy floodwater. Different letters indicate statistically significant at $p < 0.05$ (One-way ANOVA, LSD).

Figure 1 shows the growth characteristics of GXM after 96 h of incubation in paddy floodwater. The growth of GXM was significantly inhibited when NH_4^+ concentration was 13.46 mg L^{-1} ($p < 0.05$). The inhibitory rate increased with increase of NH_4^+ concentration. The growth rate of GXM was 63.8%, 50%, and 33% of the control when the NH_4^+ concentration was 13.46, 26.92, and 67.30 mg L^{-1}, respectively.

3.2 Content of phycobiliproteins

The effects of NH_4^+ stress on the content of phycobiliproteins in cells of GXM are shown in Figure 2A. Ammonium stress reduced the content of total phycobiliproteins in GXM when its concentration was ≥ 13.46 mg L^{-1} ($p < 0.05$). The intracellular contents of phycobiliproteins decreased by 14.5% - 26.3% at NH_4^+ concentrations ranged from 13.46 to 67.30 mg L^{-1}. However, there was no significant difference among the three NH_4^+ stress treatments ($p > 0.05$).

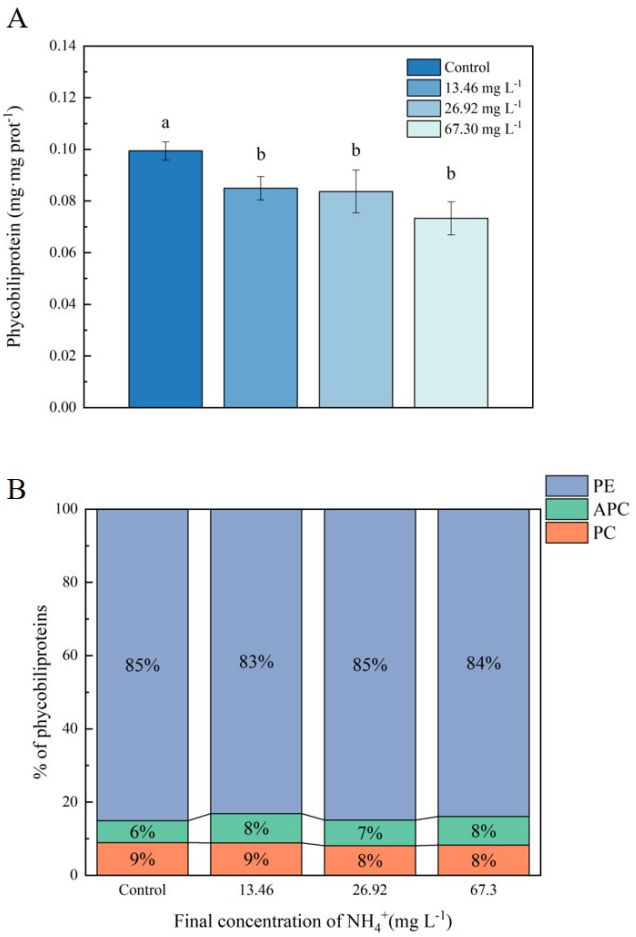

Figure 2. Effects of NH_4^+ on phycobiliproteins content (A) and phycobiliproteins constituent (B) of GXM (*N. Sphaeroides*) in paddy floodwater. Different letters indicate statistically significant at $p < 0.05$ (One-way ANOVA, LSD).

There are three kinds of phycobiliproteins in cells of GXM, namely allochthonous phycocyanin (APC), phycocyanin (PC) and phycoerythrin (PE). The effects of NH_4^+ on phycobiliproteins constituent of GXM are shown in Figure 2B. The percentage of APC in NH_4^+ stress treatments increased relative

to the control, while the PC exhibited a downward trend. The ratio of APC/PC increased significantly relative to the control. It was 132%, 131%, and 136% of the control when NH_4^+ concentration was 13.46, 26.92, and 67.30 mg L^{-1}, respectively.

3.3 Characteristics of CFT

Figure 3A shows the CFT of GXM under NH_4^+ stress in paddy floodwater. GXM exhibited a typical OJIP curve in the control and all the NH_4^+ stress treatments. Based on the characteristics of CFT, the energy distribution ratio parameter φP_0, ψ_0, φE_0, φD_0, $(1\text{-}V_k/V_j)_r$, and perform index PI_{abs} were further calculated and analyzed (Figure 3B). The results showed that the PI_{abs} of GXM in NH_4^+ stress treatments reduced by 22.3% - 35.4% ($p < 0.05$) relative to the control. The value of φP_0 was 88.4%, 90.2%, 86% of the control when NH_4^+ concentration was 13.46, 26.92 and 67.30 mg L^{-1}, respectively. The value of ψ_0 was not obviously influenced by NH_4^+ stress ($p > 0.05$). Compared with the control, the value of φE_0 decreased by 11.9%, 4.5%, and 10.5% at a NH_4^+ concentration of 13.46, 26.92, and 67.30 mg L^{-1}, respectively. The value of $(1\text{-}V_k/V_j)_r$ decreased by 4.9%-7.3% ($p < 0.05$), while φD_0 increased by 9.6%, 8.2%, and 11.7% at a NH_4^+ concentration of 13.46, 26.92, and 67.30 mg L^{-1}, respectively.

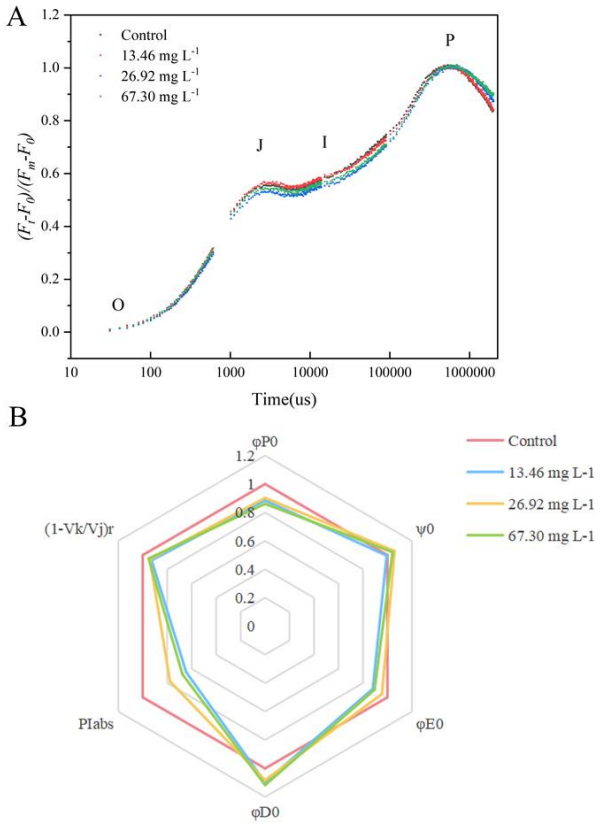

Figure 3. Effects of NH_4^+ stress on chlorophyll fluorescence transients of GXM (*N. Sphaeroides*) in paddy floodwater (A) and the parameters PI_{abs}, φP_0, ψ_0, φE_0, $(1\text{-}V_k/V_j)_r$, and φD_0 deviated from chlorophyll fluorescence transients (B).

3.4 Dinitrogen-fixing ability

As shown in Figure 4, the N_2-fixing ability of GXM was dramatically suppressed by NH_4^+ stress. The results showed that, under NH_4^+ stress, the efficiency of N_2-fixation of GXM was 11.2%, 7.6%, and 2.1% of the control at a NH_4^+ concentration of 13.46, 26.92, and 67.30 mg L^{-1}, respectively. Among the NH_4^+ addition treatments, there was no significant difference in the N_2-fixing rate between the treatments of 13.46 mg L^{-1} and 26.92 mg L^{-1}, while they were all significantly higher than that in the treatment of 67.30 mg L^{-1}.

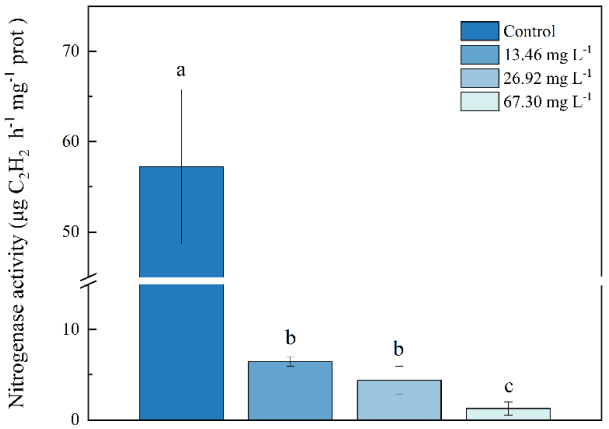

Figure 4. Effects of NH_4^+ on the activity of nitrogen fixation of GXM (*N. Sphaeroides*) in paddy floodwater. Different letters indicate statistically significant at $p < 0.05$ (One-way ANOVA, LSD).

3.5 Contents of reactive oxygen species (ROS) and malondialdehyde (MDA)

As shown in Figure 5, NH_4^+ stress significantly increased the ROS content in of cells of GXM. When the NH_4^+ concentration was $\geq$ 26.92 mg L^{-1}, the intracellular ROS significantly increased ($p < 0.05$), with an increase rate of 14.1% at 26.92 mg L^{-1} and 29.5% at 67.30 mg L^{-1}.

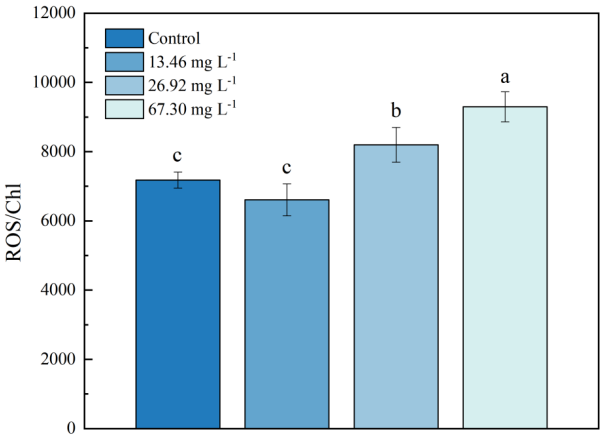

Figure 5. Effects of NH_4^+ on the content of ROS in the cells of GXM (*N. Sphaeroides*) in paddy floodwater. Different letters indicate statistically significant at $p < 0.05$ (One-way ANOVA, LSD).

The results of MDA content determination showed that the content of MDA in cells of GXM was significantly increased at a NH_4^+ concentration of 67.30 mg L^{-1}, accounting for 129.1% of the control (Figure 6). However, the content of MDA in the treatments of 13.46 and 26.92 mg L^{-1} showed no statistical difference relative to the control.

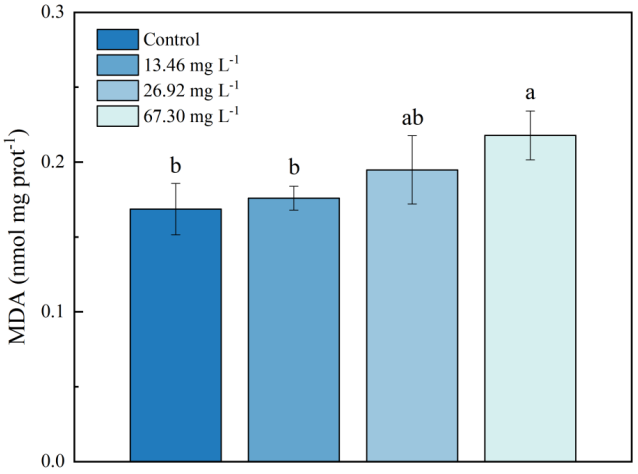

Figure 6. Effects of NH_4^+ on the content of MDA in the cells of GXM (*N. Sphaeroides*) in paddy floodwater. Different letters indicate statistically significant at $p < 0.05$ (One-way ANOVA, LSD).

3.6 Activities of Catalase (CAT)

Figure 7 shows the variations of CAT activity of GXM under NH_4^+ stress. In the paddy floodwater, the addition of NH_4^+ to a final concentration of 13.46 and 26.92 mg L^{-1} had no significant effect on activity of CAT in the cells of GXM. However, when the concentration of NH_4^+ reached 67.30 mg L^{-1}, the CAT activity in the algal cells increased significantly ($p < 0.05$), accounting for 119.4% of the control.

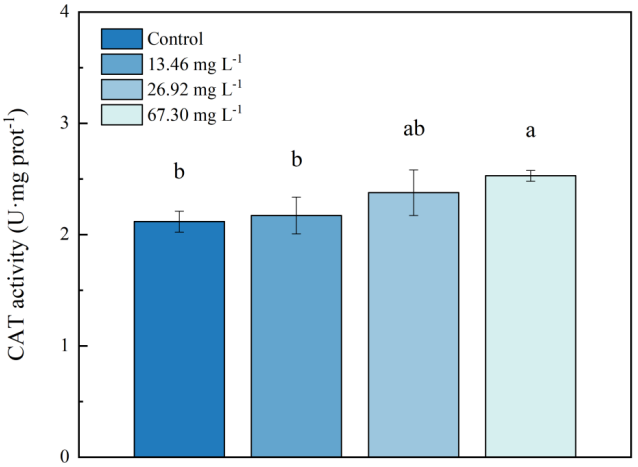

Figure 7. Effects of NH_4^+ on the activity of CAT of GXM (*N. Sphaeroides*) in paddy floodwater. Different letters indicate statistically significant at $p < 0.05$ (One-way ANOVA, LSD).

3.7 Variations of NH_4^+-N and NO_3^--N in cultural system

The variations of NH_4^+-N and NO_3^--N concentrations in cultural system were determined in this study. As shown in Figure 8, the NH_4^+-N concentration in the three NH_4^+-N addition treatments decreased along with incubation time, and it fell to below detection limit, 9.61, and 48.34 mg L^{-1} on the 96^{th} h for the NH_4^+-N addition treatment of 13.46, 26.92, and 67.30 mg L^{-1}, respectively. Opposite to the variations of NH_4^+-N, the NO_3^--N concentrations in the cultural system slightly increased after 96^{th} h of incubation.

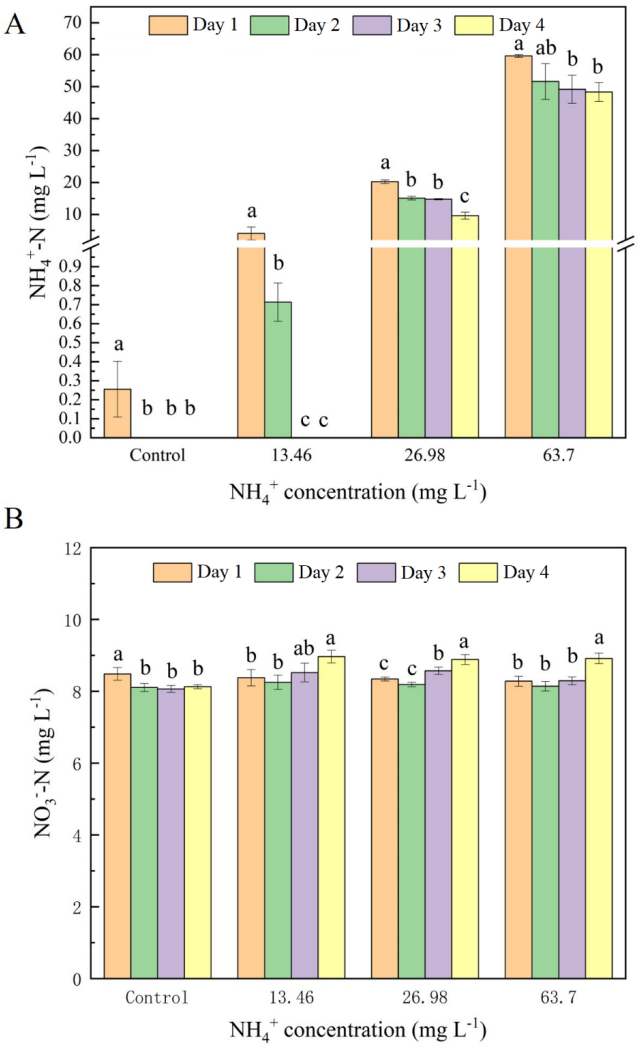

Figure 8. Variations of NH_4^+-N and NO_3^--N concentrations in the cultural system. Different letters indicate statistically significant at $p < 0.05$ (One-way ANOVA, LSD).

3.8 Variations of pH

Table 1 shows the variations of pH within the two systems, namely paddy floodwater and BG11 medium, after 24 h of incubation. Without NH_4^+ addition, the pH in the BG11 system increased by 1.26 units relative to its initial value. The

pH in the treatments of 13.46, 26.92, 67.30 mg L^{-1} in BG11 systems increased by 1.06, 0.98, and 0.77 unit, respectively. However, the pH of the paddy floodwater system showed little variations.

Table 1. Variations of pH in BG11 and paddy floodwater system

Concentration of NH_4^+	pH	
	BG11	Paddy floodwater
Control	8.21±0.09 a	7.09±0.06 a
13.46 mg L^{-1}	8.01±0.11 a	7.01±0.04 ab
26.92 mg L^{-1}	7.93±0.1 b	6.95±0.04 ab
67.30 mg L^{-1}	7.72±0.08 c	6.92±0.05 b

Note: Different letters in the table mean statistically significant.

4 Discussion

Regarding the toxic effects of NH_4^+ on diazotrophic cyanobacterium, previous studies were carried out in algal media (Dai et al., 2008). However, the original habitat of GXM is paddy fields. The physical and chemical properties of paddy floodwater are greatly different relative to those of the laboratory-formulated media. Therefore, simulating the actual growth environment of GXM may more accurately assess the toxic effects of NH_4^+ fertilizer on GXM during rice cropping.

It is reported that NH_4^+-N concentration in paddy floodwater reached 87 mg L^{-1} after 1 d of basal N fertilization at a level of 225 Kg N ha^{-1}, and the NH_4^+-N concentration maintained a level of more than 20 mg L^{-1} in the following 7 d after fertilization (Lü et al., 2022). The results presented this study showed that NH_4^+ significantly inhibited the growth of GXM at a concentration of 13.46 mg L^{-1} in paddy floodwater, suggesting that high levels of N fertilization are toxic to GXM and may result in less GXM resource in paddy fields.

Previous study investigated the toxicity of NH_4^+ on GXM in BG11 medium, Dai et al. (2008) showed that the 96-h EC_{50} of NH_4^+ on GXM in BG11 medium was 1.10 mmol L^{-1}. Similar to the report from Dai et al. (2008), a recent study in our lab showed that the 96-h EC_{50} of NH_4^+ on GXM in BG11 medium was 1.14 mmol L^{-1} (Feng, 2023). In contrast, the results presented in this study showed that the 96-h EC_{50} of NH_4^+ on growth of the GXM in paddy floodwater was 26.92 mg L^{-1} (1.496 mmol L^{-1}), indicating that, in paddy floodwater, the toxicity of NH_4^+ on GXM was weaker than that in BG11 medium.

The production of NH_3 from hydrolysis of NH_4^+ is influenced by the ambient pH. The higher of environmental pH often results in the easier hydrolysis of NH_4^+ to produce NH_3. As an un-ionised molecule, NH_3 is more likely to pass through cell membrane by free diffusion than NH_4^+. Ammonia is regarded as the main toxic form in the dissociating NH_4^+/water system (Källqvist and Svenson, 2003). Therefore, the toxicity of NH_4^+ is pH depended. The inhibitory effect of NH_4^+ on cyanobacterial photosynthetic system II is enhanced with the increase of pH (Drath et al., 2008). In this study, we determined the variation of pH in BG11 medium and paddy floodwater after inoculated GXM. Our results showed that, at a NH_4^+ concentration of 67.30 mg L^{-1}, the pH of the BG11 medium cultural system increased from 6.95 to 7.72 (Table 1), while it showed little variation in the paddy floodwater (from 6.95 to 6.92). The buffer of citric acid - sodium citrate in paddy floodwater may response for the stable pH of the paddy floodwater cultural system during incubation. It is assumed that, under a same NH_4^+ concentration, the BG11 system produces more NH_3 molecules than the paddy floodwater system, and thus is more toxic to GXM than in the paddy floodwater system. It may explain why the results from Dai et al (2008) differ from our results. In paddy field, the pH of floodwater is regulated by paddy soil. Since soil has extremely strong pH buffering characteristics, the pH of the paddy floodwater is relatively stable (Liu et al., 2020). Thus, it is speculated that the toxicity of NH_4^+ on GXM in the paddy fields may be weaker than that of in BG11 medium system.

Phycobiliproteins are important components of the photosynthetic antenna complex of cyanobacteria. They can absorb green, yellow, and orange light (450 to 650 nm) (Dagnino-Leone et al., 2022). Our results showed that NH_4^+ stress decreased phycobiliprotein content in cells of GXM, suggesting that NH_4^+ stress may decrease light energy absorption by antenna complex of cyanobacteria. APC is more efficient than PC in light collection (Lin et al., 2019). Therefore, the increase of APC/PC ratio improves light energy capture efficiency. Under NH_4^+ stress, the APC/PC ratio increased although the content of intracellular phycobiliprotein in GXM decreased. It is speculated that GXM enhanced the light-trapping efficiency of phycobiliproteins by increasing APC/PC to compensate the decrease of light absorption caused by reduction of phycobiliproteins content under NH_4^+ stress.

The variation characteristics of CFT can reflect the electron transfer characteristics of PS II in microalgae, where PI_{abs} reflects the comprehensive performance of PS II (Christen et al., 2007). As presented in Figure 3B, the PI_{abs} decreased under NH_4^+ stress, indicating that NH_4^+ stress damaged the PS II of GXM. φE_0 is the probability of electrons (after absorb light energy by reaction center) transfer to other electron acceptors located in downstream of QA in the electron transfer chain of PS II (Zagorchev et al., 2021). A decrease of φE_0 in this study indicates that NH_4^+ stress decreased the quantum yield used for electron transfer. The parameters φE_0 and ψ_0 reflect the performance of electron donor side and acceptor side of PS II reaction center, respectively (Lin et al., 2019). Our results about the variations of φP_0 and ψ_0 indicated that NH_4^+ stress in paddy floodwater damaged the electron donor side of reaction center of PS II but did not influence its acceptor side. A decrease of $(1\text{-}V_k/V_j)_r$ in this study showed that the function of the OEC of GXM was impaired under NH_4^+ stress, which was consistent to the report from Dai et

al (2008) that OEC was a target of NH_4^+ on GXM.

ROS are byproducts of aerobic metabolism (Sies et al., 2022; Jadhav et al., 2011). Under normal conditions, the production and scavenging of intracellular ROS are in dynamic balance (Yan et al., 2022; Yu et al., 2020). The results presented this study showed that the ROS and the MDA content in GXM increased significantly when the NH_4^+ concentration was 67.30 mg L^{-1}. Accordingly, the activity of CAT increased in response to NH_4^+ stress. All of these results indicate that NH_4^+ stress led to oxidative stress and oxidative damage on GXM. Photosynthetic system is the main source of ROS production for photosynthetic microorganisms (Khorobrykh et al., 2020). When the photosynthetic system is damaged by environmental stress, the high-energy electrons in the photosynthetic system, activated by light, cannot be successfully quenched by photosynthetic electron transfer, which would then be transferred to oxygen and generate ROS, leading to an increase in intracellular ROS content (Moustakas, 2022). In this study, the variational trends of intracellular ROS and MDA contents in GXM under NH_4^+ stress were consistent with the damage of its photosynthetic system. Thus, it is speculated that NH_4^+ stress interfered in electron transport of photosynthetic system of the GXM, which in turn induced an increase of intracellular ROS content and resulted in oxidative damage on GXM.

Biological N_2-fixation is a highly energy-consuming biochemical process. When the available N in environment meets the demand of diazotrophic microorganisms, they prefer to reduce the activity of nitrogenase and directly absorb available N like NH_4^+ from environment (Moisander et al., 2022). Numerous studies showed that NH_4^+ addition inhibited the activity of nitrogenase of diazotrophic microorganisms. For example, the N_2-fixation of *Crocosphaera watsonii*, a kind of diazotrophic cyanobacteria, was inhibited by 36-83% at a NH_4^+ concentration of 180 μg L^{-1} (Dekaezemacker and Bonnet, 2011). Similar reason may responsible for the decrease of N_2-fixing rate of GXM under NH_4^+ stress in this study. The results of CFT analysis showed that NH_4^+ stress impaired photosynthetic system and reduced energy production efficiency, which may be another reason for the decrease of N_2-fixing rate of GXM under NH_4^+ stress. The depression of N_2-fixing rate of GXM under NH_4^+ stress suggests that high levels of NH_4^+-based fertilization may decrease ecological role of N input of GXM in paddy field.

5 Conclusion

In this study, the toxicity of NH_4^+ on GXM (*Nostoc Sphaeroides*) in paddy floodwater was investigated. The toxic effects of NH_4^+ on GXM in paddy floodwater was weaker than that in BG11 medium, which may due to the higher pH buffering capacity of paddy floodwater than BG11 medium. Based on chlorophyll fluorescence transients analyses, both electron donor side of reaction center of PS II and oxygen evolution complex of GXM were the targets of toxicity of NH_4^+ on GXM. The damage of photosynthetic system led to the production of ROS, and resulted in oxidative damage on cells of GXM. The results presented in this study indicate that high level of NH_4^+-based fertilization may not only decrease the ecological role of N input of GXM in paddy fields, but also result in less GXM resource in paddy fileds.

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Conflict of Interest

There is no conflict of interest to be declared.

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