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Effects of different nitrogen species on sensitivity and photosynthetic stress of three common freshwater diatoms

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Abstract Different sources of nitrogen pose diverse effects to algal community, but the mechanism of inhibitory effects of nitrogen sources on freshwater diatoms is not fully understood. The purpose of this study was to compare biomass, photosynthetic activity, and morphological structure of three common freshwater diatoms (*Cyclotella meneghiniana*, *Nitzschia* sp., and *Gomphonema parvulum*) under different nitrogen sources (NO_3^- or NH_4^+). The sorption characteristic of each diatom was investigated, and chlorophyll a (Chl-a) content and oxygen evolution rate were analyzed to investigate stress of different nitrogen sources on each diatom in the batch experiments. Ammonium lowered the growth rate of *C. meneghiniana* and *Nitzschia* sp. when it was supplied in addition to growth-saturating nitrate concentrations, suggesting a combined effect of inhibition of nitrate uptake and direct ammonium stress. Oxygen evolution rate of *Nitzschia* sp. showed that the direct ammonium stress on the photosynthetic activity can be alleviated by coexistence of nitrate in the nitrogen enriched treatment, but not for *C. meneghiniana* and *G. parvulum*, which may be caused by a different nitrate transporter system within algal cells. Transmission electron microscopy was used to assess the toxicity of ammonium on ultrastructural chloroplast of each diatom. Ultrastructural changes in chloroplasts showed undefined electron-dense granules and lipid droplets, but the membrane integrity of cell was maintained, suggesting an adaptation to adjustment to ammonia stress. Results showed that *Cyclotella meneghiniana* and *Nitzschia* sp. were more sensitive to ammonium stress than *Gomphonema parvulum* on growth, but the mechanism remains unclear.

Keywords Diatoms · Nitrogen · Sensitivity · Chloroplast · Stress

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Introduction

The outbreak of cyanobacteria blooms in Taihu Lake has attracted worldwide attention on the eutrophication problems in China (Guo 2007). Ammonia concentration in Taihu Lake reached 1.77 mg/L before cyanobacteria blooms (Zhang et al. 2011), and ammonia contamination observed in channel

segment of Grand Canal at the downstream of Taihu Lake was significantly higher than any other part, with a maximum concentration of 7.18 mg/L during the wet season (Wang et al. 2010). Dai et al. (2012) experimentally compared more than a dozen common phytoplankton species' responses to ammonia stress and suggested that phytoplankton species succession in shallow lakes may be associated with their tolerance to ammonia stress. The high concentration of ammonia, typically observed in spring in shallow lakes in China (the average and maximum ammonia concentration were 1.12 mg/L, and 10.08 mg/L), may inhibit the growth of some bloom-forming cyanobacteria taxa (e.g., *Microcystis aeruginosa*), while the same nutrient in low concentration in summer is often associated with cyanobacteria blooms (Dai et al. 2012; Ni et al. 2012). Nitrate can also enhance as well as inhibit the growth of *M. aeruginosa* and reduce its photosynthesis under certain high concentrations (from 49.98 to 300.02 mg/L) (Chen et al. 2009). In contrast, stress of different nitrogen sources on diatoms is not as well known as cyanobacteria (but see Dai et al. 2012). The use of diatoms as water quality indicators has been reported by a number of earlier studies (Quinlan et al. 2011). The nutrient conditions of different water bodies could have a distinct effect on the abundance of specific diatom species, because tolerance differences exist among various species (Bennion et al. 1996; Leland et al. 2001). For instance, *Gomphonema parvulum* was found to be dominant in hypereutrophic lakes, *Melosira varians* and *Synedra* spp., dominated the community in eutrophic water bodies (Pei et al. 2010), and *M. varians* was sensitive to organic water pollution (Szczepocka and Szulc 2009).

Few studies paid attention to the effects of physical and chemical parameters on photosynthetic activities of diatoms. Recently, ammonia (NH_3) has been confirmed as an inhibitory of O_2 evolution at alkaline pH, by interacting with carboxylate groups coupled to the Mn_4CaO_5 cluster as direct ligands or proton transfer mediators, causing inhibition of O_2 evolving reaction in the center of Photosystem II(Tsuno et al. 2011; Hou et al. 2011). However, an understanding of the effect of ammonia on the photosynthetic rate of various freshwater diatoms remains unknown. Clonal cultures of algae are widely used in laboratory experiments and have contributed greatly to our knowledge of microbial ecosystems (Thessen et al. 2009). Among the multitude of diatom species, *Gomphonema parvulum* and *Cyclotella meneghiniana* represent the most widely distributed species (Swift and Wheeler 1991; Rimet et al. 2009), both *G. parvulum* and *C. meneghiniana* have been frequently observed as dominant species in eutrophic water bodies (Finlay et al. 2002; Rimet et al. 2009; Pei et al. 2010), *C. meneghiniana* has even been studied as a structure template in material science (Lenoci and Camp 2008). Because many physiological characteristics vary drastically among different species, *Nitzschia* sp., *G. parvulum,* and *C. meneghiniana*, all dominants in Chinese water bodies were chosen as representatives to investigate the effect of ammonia on photosystem and O_2 evolving reaction of freshwater diatoms.

The purpose of this study was to compare sensitivity of three common freshwater diatoms (*Cyclotella meneghiniana*, *Nitzschia* sp. and *Gomphonema parvulum*) by measuring their sorption behavior, photosynthetic activity, and ultrastructure under different nitrogen sources (NO_3^- and NH_4^+). O_2 evolving rate was employed as an indicator of the toxicity of ammonium on the photosystem of each diatom, and transmission electron microscopy (TEM) was further used to assess the ultrastructural chloroplast changes of each diatom under ammonia stress.

Experiments

Cultivation of algae

Nitzschia sp., *Gomphonema parvulum,* and *Cyclotella meneghiniana* were obtained from the Freshwater Algae Culture Collection of the Institute of Hydrobiology, the Chinese Academy of Sciences (Wuhan, Hubei, P. R. China). All experiments were carried out on batch cultures of *Nitzschia* sp., *G. parvulum,* and *C. meneghiniana*, grown in a modified form of D1 medium, containing 20.0 mg/L nitrogen in the form of either $NaNO_3$ or NH_4Cl, or 20.0 mg/L $NaNO_3$ and 20.0 mg/L NH_4Cl. For the control treatment, the medium was prepared with NaCl only. As the standard D1 medium contains 20.0 mg/L nitrogen in the form of $NaNO_3$, this concentration was used to set up the experiments. All batch experiments were performed in 500 mL Erlenmeyer flasks in triplicate for each treatment. These three selected species were inoculated into the experimental flasks with 200 mL

autoclaved diatom D1 medium and maintained at 22 °C under a photon flux density of 40 μmol photons/m^2/s, with a cycle of 12 h light : 12 h dark. The initial cell concentration of each diatom in the batch cultures was about 5×10^4 cells/mL. Exponentially growing cultures in D1 medium, at a cell density about 50×10^6 cells/mL, were pelleted by centrifugation at 4,000 rpm for 15 min, washed twice with nitrogen-free medium, and then transferred to an equal volume of different nitrogen treated medium, respectively (Table 1).

Measurements of chlorophyll a and nitrogen concentrations

Samples for Chl-a measurements under various nitrogen sources were collected every 3 days from the batch cultures, with a study period of 15 days. An aliquot of 15 mL were filtered through 0.45 μm Whatman AH glass fiber filters, held frozen overnight prior to extraction using ethanol (Jespersen and Christoffersen, 1987). Chl-a was measured spectrophotometrically at 665 nm and 750 nm using a Shimadzu UV2450 spectrophotometer.

Samples for nitrogen (nitrate/nitrite/ammonia) measurements under various nitrogen sources were collected every 3 days from the batch cultures, with a study period of 15 days. An aliquot of 5 mL were filtered through 0.45 μm Whatman AH glass fiber filters, and the concentration of nitrate/nitrite/ammonia in the medium was then measured using a HACH DR890 analyzer (Ni et al. 2012).

Measurements of photosynthetic oxygen evolution

Effects on the photosynthetic activity of three typical freshwater diatoms were determined using a Clark-type oxygen electrode (Oxygraph, Hansatech, UK) at 22 °C with 40 μmol photons/m^2/s of photosynthetically active radiation (Petit et al. 2010). Two mL of the fivefold concentrated algal suspension was transferred into an electrode unit, and oxygen evolution rate (nmol/mL/min) was determined according to the detected signal. Prior to each measurement, the samples were adapted for 2 min before the oxygen evolution rate reached a stable level, and then the increase in oxygen concentration was recorded during the next 10 min. For each measurement, the result was corrected for biomass of viable cells and expressed as μmol mg/(Chl-a)/h.

Characterization of ultrastructure of chloroplast

Double fixation

The specimen was first fixed with 2.5 % glutaraldehyde in phosphate buffer (0.1 M, pH 7.0) for more than 4 h then washed three times in the phosphate buffer for 15 min, respectively. The specimen was then postfixed with 1 % OsO_4 in the phosphate buffer for 1 h and washed three times in the phosphate buffer for 15 min, respectively.

Dehydration

The specimen was first dehydrated by a graded series of mixed acetone and water (50, 70, 80, 90, 95 and 100 %) for about 15–20 min, respectively, then transferred to pure acetone for 20 min.

Infiltration: The specimen was placed in 1:1 mixture of absolute acetone and the final Spurr resin mixture for 1 h at room temperature then transferred to a 1:3 mixture of absolute acetone and the final resin mixture for 3 h and to the final Spurr resin mixture for overnight.

Table 1 Nitrogen concentration in each treatment for three common freshwater diatoms

Diatom species	Nitrogen concentration in each treatment (mg/L)			
	Control (NaCl)	D1 medium ($NaNO_3$)	Ammonium treatment (NH_4Cl)	Nitrogen enriched treatments ($NaNO_3$ + NH_4Cl)
Gomphonema parvulum	Nitrogen-free D1 medium (NaCl replaced $NaNO_3$)	Standard D1 medium (20 mg/L NO_3-N)	Modified D1 medium (20 mg/L NH_4-N)	Enriched D1 medium (20 mg/L NO_3-N + 20 mg/L NH_4-N)
Cyclotella meneghiniana				
Nitzschia sp.				

Embedding and ultrathin sectioning

The specimen was placed in eppendorf contained embedding and heated at 70 for about 9 h. The specimen sections were stained by uranyl acetate and alkaline lead citrate for 15 min, respectively, and observed in TEM of Model JEM-1230.

Data analysis

Three replicates were used in each experiment, and all the data were expressed as mean ± SD ANOVA was performed for each diatom to determine whether an overall significant response to different nitrogen sources was observed. If the response was significant, a multiple comparison using Turkey Highly Significant Difference test was performed for each diatom to determine which specific nitrogen source has significant effects on Chl-a and oxygen evolution rate. The data in the figures were presented with the mean ± SD.

Results

Diatom sensitivity to ammonium and nitrate

Cyclotella meneghiniana, *Gomphonema parvulum,* and *Nitzschia* sp. showed a little difference on the uptake preference to different nitrogen source (NH_4^+ and NO_3^-). Nitrate concentration in the ammonium treatment and the control treatment showed no significant difference for both *C. meneghiniana* and *G. parvulum* ($P > 0.05$), and no significant difference on ammonia concentration was observed between the control treatment and D1 medium for all the treatments of *C. meneghiniana*, *G. parvulum,* and *Nitzschia* sp. ($P > 0.05$). But, nitrate concentration showed no significant difference between the nitrogen enriched treatments and in D1 medium of *C. meneghiniana* and *Nitzschia* sp. ($P > 0.05$) (Table 2), and ammonia concentration in the nitrogen enriched treatments of *C. meneghiniana* and *Nitzschia* sp. was statistically higher than that of the ammonium treatments ($P < 0.01$), suggesting that nitrate was the first nitrogen source for *C. meneghiniana* and *Nitzschia* sp., and nitrate had a stimulatory effect of ammonia uptake on both *C. meneghiniana* and *Nitzschia* sp.. Nitrate concentration in the nitrogen enriched treatments of *G. parvulum* was statistically higher than that of D1 medium ($P < 0.01$) (Table 2), and ammonia concentration in the nitrogen enriched treatments of *G. parvulum* was statistically higher than that of the ammonium treatments ($P < 0.01$), suggesting that nitrate was the first nitrogen source for *G. parvulum*, and ammonia had an inhibitory effect of nitrate uptake on *G. parvulum*.

The growth of *Nitzschia* sp., *G. parvulum* and *C. meneghiniana* had a similar pattern under the nitrogen limited conditions. Chl-a concentration in D1 medium, the ammonium and nitrogen enriched treatments was statistically higher than that of the control (paired *t* test, $p < 0.01$). Ammonium did not support the growth of *C. meneghiniana* at concentrations higher than 20 mg/L, and even lowered the growth rate, when it was supplied in addition to growth-saturating nitrate concentrations. Chl-a concentration of *C. meneghiniana* in D1 medium was statistically higher than that of the nitrogen enriched and ammonium treatments. This seemed to be a combined effect of inhibition of nitrate uptake and direct ammonia stress on *C. meneghiniana*.

Chl-a concentrations of *G. parvulum* and *Nitzschia* sp. in D1 medium were statistically higher than those of the ammonium treatments ($P < 0.05$). Chl-a concentrations of *G. parvulum* and *Nitzschia* sp. showed no significant difference between the nitrogen enriched

Table 2 Mean nitrate and ammonia concentration differences between two treatments in the batch experiments

Diatom species	Nitrate (mg/L)		Ammonia (mg/L)	
	NH_4^++ NO_3^- versus NO_3^-	NH_4^+ versus control	NH_4^++ NO_3^- versus NH_4^+	NO_3^- versus control
Gomphonema parvulum	0.008**	n.s.	0.000**	n.s.
Cyclotella meneghiniana	n.s.	n.s.	0.000**	n.s.
Nitzschia sp.	n.s.	0.025*	0.000**	n.s.

P values for paired t tests are presented. *P* values greater than 0.05 are denoted as n.s. (not significant)

* $p < 0.05$, ** $p < 0.01$

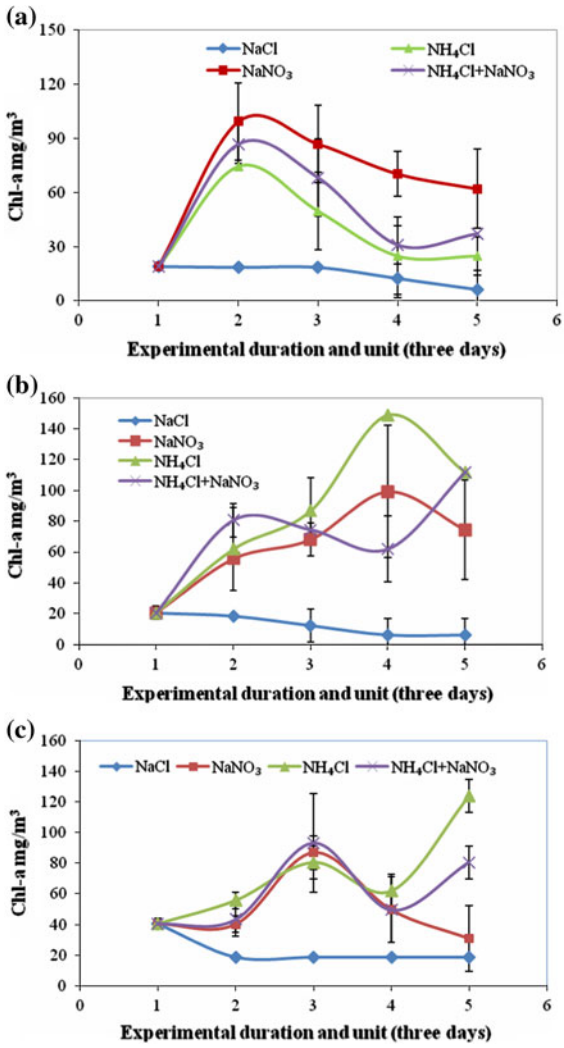

Fig. 1 Chl-a concentrations of *Cyclotella meneghiniana* (**a**), *Gomphonema parvulum* (**b**) and *Nitzschia* sp. (**c**) under different treatments

and ammonium treatments, and as well as between the nitrogen enriched treatments and D1 medium. It seemed that *G. parvulum* and *Nitzschia* sp. are more tolerant to ammonium than *C. meneghiniana* (Fig. 1).

Response of photosynthetic rate to ammonia stress

Nitzschia sp., *G. parvulum,* and *C. meneghiniana* had a little different pattern under ammonia stress. Oxygen evolution rate of *C. meneghiniana* in D1 medium was 79.57 μmol/mg/Chl-a/h, statistically higher than any other treatments in the experiments ($P < 0.05$) (Fig. 2a). Oxygen evolution rate of *C. meneghiniana* in the nitrogen enriched treatment was statistically higher than that of the ammonium treatment ($P < 0.05$), this seemed to be a direct ammonia stress on the photosynthetic activity of *C. meneghiniana*, and the effect can be alleviated by coexistence of nitrate in the nitrogen enriched treatment. Oxygen evolution rate of *C. meneghiniana* in the nitrogen enriched and ammonium treatments was significantly higher than that of the control ($P < 0.01$), but no difference was observed between the nitrogen enriched and ammonium treatments. This seemed to be a direct ammonia stress on the photosynthetic activity of *C. meneghiniana*, and the effect cannot be alleviated by coexistence of nitrate in the nitrogen enriched treatment.

Oxygen evolution rate of *G. parvulum* in D1 medium was 65.49 μmol/mg/Chl-a/h, significantly higher than any other treatments in the experiments ($P < 0.01$) (Fig. 2b). Oxygen evolution rate of *G. parvulum* in the nitrogen enriched and ammonium treatments was significantly higher than that of the control ($P < 0.01$), but no difference was observed between the nitrogen enriched and ammonium treatments. This seemed to be a direct ammonia stress on the photosynthetic activity of *G. parvulum*, and the effect cannot be alleviated by coexistence of nitrate in the nitrogen enriched treatment.

Oxygen evolution rate of *Nitzschia* sp. in D1 medium was 79.24 μmol/mg Chl-a/h, significantly higher than any other treatments in the experiments ($P < 0.01$) (Fig. 2c). Oxygen evolution rate of *Nitzschia* sp. in the nitrogen enriched and ammonium treatments was significantly higher than that of the control ($P < 0.01$), and oxygen evolution rate of *Nitzschia* sp. in the nitrogen enriched treatment was statistically higher than that of the ammonium treatment ($P < 0.05$), this seemed to be a direct ammonia stress on the photosynthetic activity of *Nitzschia* sp., and the effect can be alleviated by coexistence of nitrate in the nitrogen enriched treatment.

Ultrastructure changes of chloroplast to ammonia stress

Normal cell organelles of *Nitzschia* sp. were distinguishable except for the nucleus under TEM, and the cytoplasm became vacuolated (Fig. 3b). Figure 3c showed a crescent-moon shaped chloroplast after 15 days growth under D1 medium. Some undefined electron-dense granules were observed in the chloroplast

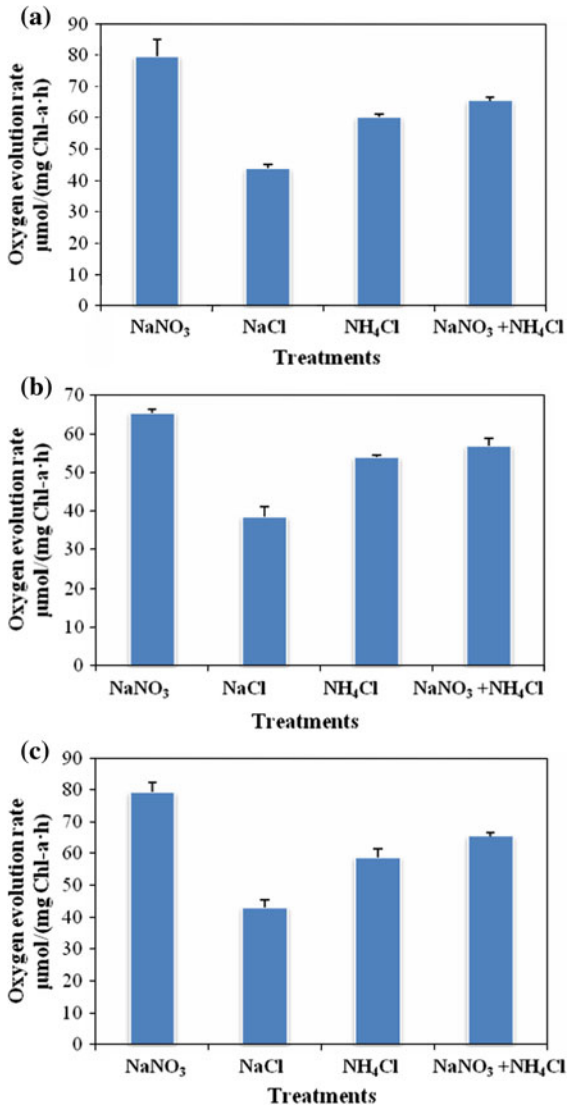

Fig. 2 Oxygen evolution rate of *Cyclotella meneghiniana* (**a**), *Gomphonema parvulum* (**b**) and *Nitzschia* sp. (**c**) under different treatments

(Fig. 3a). The Grana of *Nitzschia* sp. were compacted in the chloroplasts of all the treatments except the control (Fig. 4a–d), and lipid droplets were observed in most cells of the nitrogen enriched treatments.

Ultrastructural changes of the chloroplast in *G. parvulum* under the different treatments after 15 days were shown in Fig. 5. The cytoplasm of *G. parvulum* became vacuolated under TEM (Fig. 5a). The grana of *G. parvulum* were not compacted in the chloroplasts of the ammonium and nitrogen enriched treatment when compared to the chloroplasts in D1 medium (Fig. 5b, c, d).

Discussion

Sensitivity difference of typical freshwater diatoms on ammonia and nitrate

Freshwater diatoms showed different sensitivities on nitrogen uptake and ammonia stress. *Gomphonema parvulum* and *Nitzschia* sp. are more tolerant to ammonium than *Cyclotella meneghiniana*, as the Chl-a concentration of *Gomphonema parvulum* and *Nitzschia* sp. showed no significant difference between the nitrogen enriched treatments and D1 medium. Similar results reported by Kutka and Richards (1997) showed *C. meneghiniana* responded negatively and *G. parvulum* responded positively to ammonia diffusing pots in the field studies in a Minnesota river basin. Rushforth et al. (1981) also confirmed *G. parvulum* exhibited a better performance with a high level of ammonia, while *C. meneghiniana* was stimulated by a high level of nitrate.

The Chl-a concentration of *C. meneghiniana* in D1 medium was statistically higher than that of the nitrogen enriched and ammonium treatments ($P < 0.05$), but nitrate concentrations showed no significant difference between the nitrogen enriched treatment and in D1 medium of *C. meneghiniana* ($P > 0.05$) (Table 2). This suggested that nitrate was the first nitrogen source for *C. meneghiniana*, and ammonia turned out to have a direct stress on *C. meneghiniana*. Nitrate was also the first nitrogen source for *G. parvulum*, and ammonia has an inhibitory effect of nitrate uptake on *G. parvulum*, as the nitrate concentration in the nitrogen enriched treatment of *G. parvulum* was statistically higher than that of D1 medium ($P < 0.01$) (Table 2), and the ammonia concentration in the nitrogen enriched treatment of *G. parvulum* was statistically higher than that of the ammonium treatment ($P < 0.01$), but the Chl-a concentration of *G.parvulum* and *Nitzschia* sp. in D1 medium was statistically higher than that of the ammonium treatment ($P < 0.05$). Two distinct nitrate transport systems have been confirmed, one operating as a nitrate-proton symporter while the other as an energy-independent nitrate/nitrite antiporter (Wood et al. 2002). The mechanism of ammonia influencing nitrate uptake was either through the feedback regulation of nitrate metabolism (Zhuo et al. 1999; Orsel et al. 2002) or through the direct ion effect on the membrane potential (Ayling 1993). The cotransporter

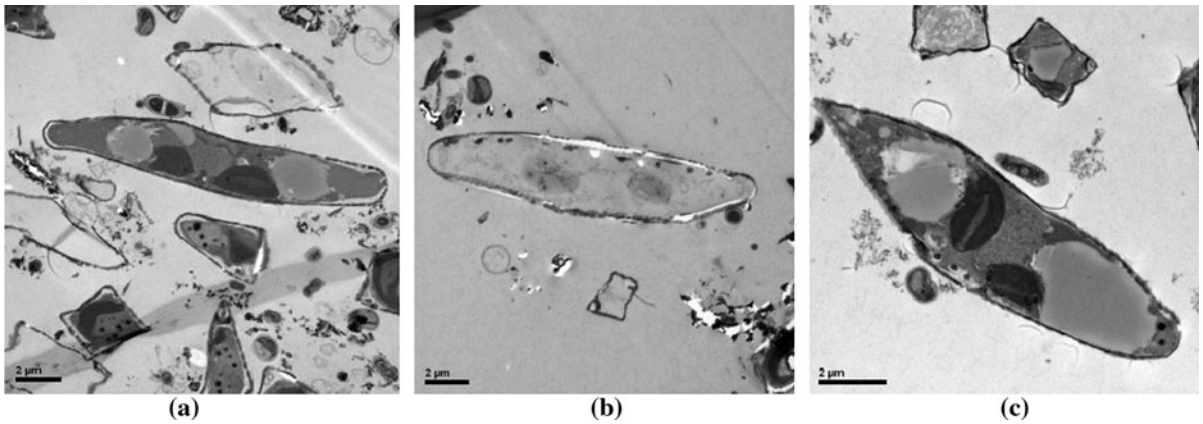

Fig. 3 Ultrastrucatural changes of *Nitzschia* sp. under treatments of ammonium (**a**), NaCl (**b**) and nitrate (**c**)

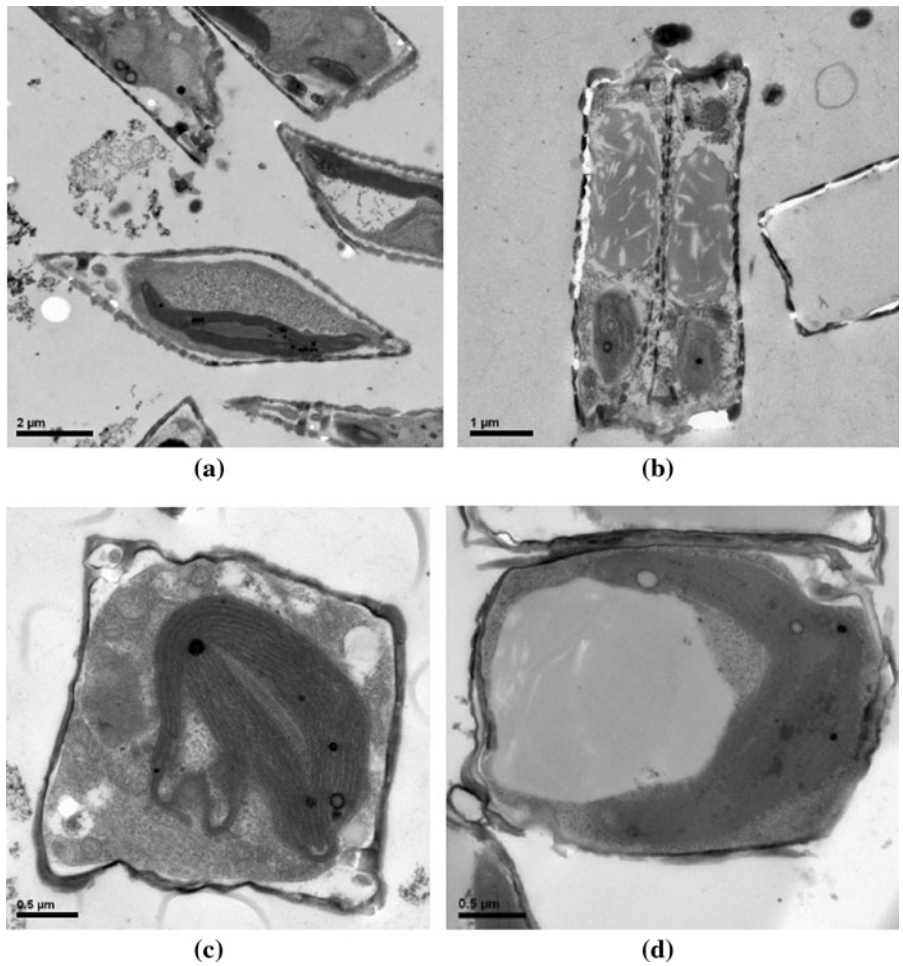

Fig. 4 Ultrastrucatural changes of chloroplast in *Nitzschia* sp. under treatments of ammonium (**a**), NaCl (**b**), nitrate (**c**) and ammonium + nitrate (**d**)

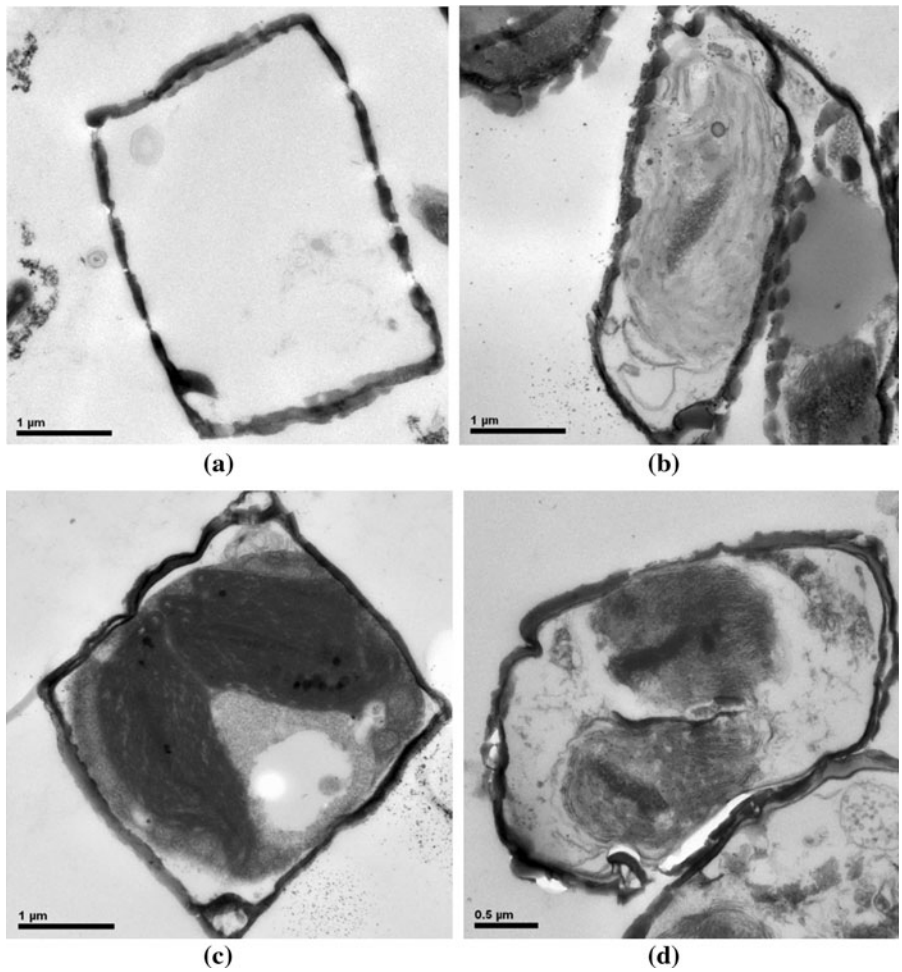

Fig. 5 Ultrastrucatural changes of chloroplast in *Gomphonema parvulum* under treatments of ammonium (**a**), NaCl (**b**), nitrate (**c**) and ammonium + nitrate (**d**)

of nitrate and proton involves H^+-ATPase, proton cross-membrane gradient, membrane potential difference, and other membrane characteristics (Colmer and Bloom 1998; Crawford and Glass 1998).

The pathway of nitrate assimilation in diatoms consists of two parts, and nitrate is taken up into the diatom cell first, reduced to nitrite by NADH-dependent nitrate reductase (NR) (Allent et al. 2005), nitrite then is transported into the chloroplast and further reduced to ammonium by nitrite reductase (NiR) (Bowler et al. 2010). Under the growth-saturating ammonia concentrations, NR in *G. parvulum* was still activated, as the nitrite concentration in the nitrogen enriched treatment was statistically higher than that of the ammonium treatment ($P < 0.01$) (Table 3).

Effects of ammonia stress on photosynthetic rate

In a natural environment, flux of nutrients from the water column to the sediment occur to an important extent by sinking of autotrophic cells, especially by diatoms (Stehfest et al. 2005). Meanwhile, considering the great contribution of diatoms to global carbon fixation (Hurtley 2009), the factors that may affect the diatom photosynthetic efficiency deserve much more attentions. In our study, the photosynthetic activities of typical freshwater diatoms showed a little different pattern under ammonia stress. Oxygen evolution rate of *C. meneghiniana* in D1 medium was statistically higher than that of any other treatments in the experiments ($P < 0.05$) (Fig. 2a). Oxygen evolution rate of *G. parvulum* and *Nitzschia* sp. in D1 medium

Table 3 Mean nitrite concentration differences between two treatments in the batch experiments

Diatom species	Nitrite (mg/L)					
	NO_3^- versus $NH_4^+ + NO_3^-$	$NH_4^+ + NO_3^-$ versus control	$NH_4^+ + NO_3^-$ versus NH_4^+	NO_3^- versus control	NO_3^- versus NH_4^+	NH_4^+ versus control
Gomphonema parvulum	0.000**	0.001**	0.001**	0.000**	0.000**	n.s.
Cyclotella meneghiniana	n.s.	n.s.	0.012*	n.s.	0.004**	n.s.
Nitzschia sp.	n.s.	0.027*	n.s.	0.003**	n.s.	0.009**

P values for paired *t* tests are presented. *P* values greater than 0.05 are denoted as n.s. (not significant)

* $p < 0.05$, ** $p < 0.01$

was both significantly higher than that of any other treatments in the experiments ($P < 0.01$) (Fig. 2b, c). When ammonia was supplied in addition to growth-saturating nitrate concentrations for *Nitzschia* sp., the direct ammonia stress on photosynthetic activity can be alleviated by coexistence of nitrate in the nitrogen enriched treatment, as oxygen evolution rate of *Nitzschia* sp. in the nitrogen enriched treatment was statistically higher than that of the ammonium treatment ($P < 0.05$). But, *G. parvulum* or *C. meneghiniana*, showed no difference between the nitrogen enriched and ammonium treatments ($P > 0.05$), although oxygen evolution rate of both diatoms in the nitrogen enriched and ammonium treatments was significantly higher than that of the control ($P < 0.01$), indicating ammonia caused potentially more damage to *G. parvulum* or *C. meneghiniana* than *Nitzschia* sp.

Different species of diatoms respond differently to metal, organic pollution, eutrophication, ionic strength, and other environmental variables because of differences in their physiological tolerances (Gold et al. 2003). *C. meneghiniana* and *G. parvulum* have been described as high pollution-tolerant species in many studies (Morin et al. 2008; Duong et al. 2010), and when *G. parvulum* was transferred to unpolluted sites, its abundance declined (Rimet et al. 2009). *G. parvulum* was found to be tolerant to high levels of cadmium, and in contrast, *C. meneghiniana* was sensitive to cadmium pollution (Duong et al. 2008). However, in this study, *Nitzschia* sp. appeared to be more tolerant to ammonia stress than *G. parvulum* or *C. meneghiniana*. The different responses of pennate diatoms (*G. parvulum* and *Nitzschia* sp.) to ammonia stress may associate with their varied ultrastructure. *C. meneghiniana* is a small discoid diatom that becomes relatively elongate and cylindrical as the valve diameter decreases during vegetative division, and the chloroplasts are surrounded by a layer of chloroplast endoplasmic reticulum (Hoops and Floyd 1979).

The mechanism of ammonia as an inhibitory of O_2 evolution on higher plants has recently been confirmed, by binding directly to the Mn site and replacing one of the bridging oxygen atoms on the core structure of Mn_4CaO_5 cluster in the Photosystem II. But, the mechanism of ammonia stress on the photosystem of diatoms remains unclear to our knowledge.

Effects of ammonia stress on ultrastructure of chloroplast

Normal diatom cells have similar morphological features. Chloroplasts are enveloped by chloroplast endoplasmic reticulum; pyrenoids are traversed by a single thylakoid; mitochondria contain tubular cristae (Hoops and Floyd 1979). Environmental stress may cause organelles disorganization and membrane disruption of cells (Lage-Pinto et al. 2008), and ultrastructural changes on chloroplast, such as deceased size accompanied by electron-dense stroma, thylakoids disorganization can be indicators for photosynthesis inhibition (Kivimaenpaa et al. 2004). Normal cell organelles of all three freshwater diatoms were distinguishable except for the nucleus under TEM, and the cytoplasm became vacuolated under nitrogen depletion treatments. Grana of *G. parvulum* were not compacted in the chloroplasts of the ammonium and nitrogen enriched treatments when compared to chloroplasts in D1 medium (Fig. 5b, c, d).

Crescent-moon shape chloroplast under D1 medium was showed in Fig. 5c, while some undefined electron-dense granules were observed in the chloroplast under the ammonia treatment of *Nitzschia* sp.

(Fig. 3a). Grana of *Nitzschia* sp. were compacted in the chloroplasts of all the treatments except the control (Fig. 3a–d), and lipid droplets were observed in most cells of the nitrogen enriched treatment, but no thylakoids disorganization were recorded in all the treatments.

Conclusion

Gomphonema parvulum was more tolerant to ammonia stress than *Cyclotella meneghiniana* and *Nitzschia* sp. on growth. A combined effect of inhibition of nitrate uptake was found for *C. meneghiniana* and *Nitzschia* sp.. Direct ammonia stress on the photosynthetic activity of *Nitzschia* sp. can be alleviated by coexistence of nitrate in the nitrogen enriched treatment, which may be caused by different nitrate transporter system within algal cells. TEM results confirmed ultrastructural changes of *Nitzschia* sp. in chloroplast, with undefined electron-dense granules and lipid droplets, but the membrane integrity of cell was maintained, suggesting an adaptation of typical freshwater diatoms to adjustment to ammonia stress.

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