The influence of macronitrogen (NO$_3^-$ and NH$_4^+$) addition with *Ulva pertusa* on dissolved inorganic carbon system

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Abstract

The influence of macronitrogen (NO$_3^-$ and NH$_4^+$) addition with *Ulva pertusa* on dissolved inorganic carbon system in seawater was studied. The results indicate that p(CO$_2$) and HCO$_3^-$ concentration decrease significantly, while pH and CO$_3^{2-}$ concentration increase significantly. When the concentration of NO$_3^-$ was less than 71 μmol/dm^3 or NH$_4^+$ was less than 49.7 μmol/dm^3, dissolved inorganic carbon (DIC) absorption rates by *Ulva pertusa* generally increased with the increasing of nitrogen concentration. The DIC decreased 151 μmol/dm^3 with the addition of 71 μmol/dm^3 NO$_3^-$ and decreased 232 μmol/dm^3 with the addition of 49.7 μmol/dm^3 NH$_4^+$ after the experiment compared with DIC measured without nitrogen addition. A significant negative-correlation was found between Δc(DIC) and growth rate (μ) of *Ulva pertusa* ($r = -0.91$, $P < 0.000\,1$, $n=11$). NH$_4^+$ had more influence on the species of inorganic carbon system than NO$_3^-$.

Key words: dissolved inorganic carbon, algae, simulated experiments, *Ulva pertusa*, macronitrogen, the Jiaozhou Bay

1 Introduction

In many estuarine and coastal waters, eutrophication has become a serious environmental problem. Eutrophication as a result of anthropogenic input of nutrients is characterized by high seawater nutrient concentrations and low water transparency, both of which have great impact on ecosystem structure and function, including algal growth, biomass and species composition, etc. (Livingston, 2002). In some extreme cases macronutrient may lead to the development of massive phytoplankton blooms as well as algal blooms (Beman et al., 2005). As all we known, biological CO$_2$ fixation by photosynthesis can decrease the concentration of CO$_2$. Thus, macronutrient might also affect the DIC cycling in seawater. In the past few decades, there have been several studies of the interactions between macronutrient and marine phytoplankton, between dissolved inorganic carbon system and the marine phytoplankton and between metal bioaccumulation in phytoplankton and macronutrient (Zheng et al., 2009; Hlaili, 2006; Sharon and Kahara, 2003; Masao et al., 2002; Rainbow and Black, 2002). These studies indicate that the concentration of DIC and macronutrient critically affect the biomass of macroalgae and metal uptake rate in phytoplankton. Recently, more and more attention has been devoted to the study of effects of DIC availability on nutrient uptake of algae. Huertas et al. (2000) indicated that the uptake of NO$_3^-$ and PO$_4^{3-}$ by *N. maculata* was dependent on the inorganic carbon level.

The total amount of DIC is the main forms of carbon in seawater and plays an important role in the carbon cycle (Zhang et al., 2009; Zeebe and Wolf-Gladrow, 2001). The DIC in water includes CO$_2$ (free CO$_2$+H$_2$CO$_3$), HCO$_3^-$ and CO$_3^{2-}$. All aquatic plants

use CO_2 for photosynthesis and some species use HCO_3^- (Zhi et al., 2008; Raven, 1991). The availability of HCO_3^- and CO_2 is variable in water habitats and the DIC concentration can be subjected to large variations when biological activity is high. Thus, DIC is very important to the growth of algae as well as nutrient. However, there has been a particular lack of studying the influence of macronutrients on dissolved inorganic carbon system changed by macroalgae and subsequent transfer as well as the interaction of DIC uptake by algae and nutrient.

In this study, we first investigated the influence of macronutrient additions coupled with macroalgae *Ulva pertusa* on the component transfer of inorganic carbon system and change of air-sea CO_2 flux. We considered two forms of nitrogen (N)-nitrate (NO_3^-) ammonium (NH_4^+) in this study. Our objectives were to test how the inorganic carbon system changed un-

der macronutrient additions and whether there was any correlation in *Ulva pertusa* growth rate and DIC and nutrient level.

2 Materials and methods

2.1 *Plant material and cultivation*

The *Ulva pertusa* was collected from the lower intertidal rock at low tide in June 2006, along the coast of Bada guan (36°02′N, 120°16′E), the Jiaozhou Bay in China. Plants were cleaned and washed off visible epiphytes and any accumulated sediments, and then placed in boxes filled with filtered water with Whatman GF/C fiber membrane from the bay. After 7 d of laboratory maintenance, the healthy individuals were selected for use in subsequent experiment. The background values of the Jiaozhou Bay seawater were measured (Table 1).

Table 1. Background values of the sampled seawater

	Parameter					
	Temperature/°C	pH	S	NO_3^- concentration/ $\mu mol \cdot dm^{-3}$	NH_4^+ concentration/ $\mu mol \cdot dm^{-3}$	Seawater transparency/m
Backgroundvalue	21.2±0.2	8.012±0.002	31.14±0.01	7.1±0.04	4.97±0.02	1.8

2.2 *Laboratory experimental set-up*

The experiment was conducted in acid-cleaned polycarbonate boxes (L: 50 cm, W: 40 cm, H: 30 cm) containing 40 L of filtered seawater collected from the same site and maintained for 24 h in order to maintain DIC concentration in equilibrium with air after DIC increase due to water mixing with air related to filtration (initial CO_2 concentration about 2 179 $\mu mol/dm^3$). Then, approximately (14.520±0.004) g (fresh mass) (3.521 g dry mass) of *Ulva pertusa* was introduced to each box. For each treatment two boxes were used. A subset of the boxes was then incubated in the laboratory at room temperature (20±1)°C for 9 d.

There were three treatments: NO_3^- addition ($+NO_3^-$), NH_4^+ addition ($+NH_4^+$) and no addition (C, control). NO_3^- and NH_4^+ were added as KNO_3 and NH_4Cl, respectively. Each enrichment treatment had five nutrient levels, both were 2, 5, 10, 50 and 100 times higher than the natural nutrient concentration. In the sea, because pH is strongly buffered (Stumm and Morgan, 1981), the addition of nutrient has little impact on pH at the beginning (P >0.05).

2.3 *Determination of DIC, concentratin nutrient, growth rate and flux of carbon dioxide*

Total alkalinity (Talk) was measured by the potentiometric titration at (25±0.05)°C with a PC-controlled titration cell. The end points were determined by the Gran function with a precision of 0.1%. The measurement of pH was at (25±0.05)°C by a Ross combination electrode (Orion), calibrated on the total hydrogen ion concentration scale, using the NBS buffers. The precision of the pH measurement was estimated to be ±0.004 pH. The concentration of DIC was calculated from the pH and Talk measurements with the dissociation constants of carbonic acid from Roy et al. (1993), the ratio of borate molality obtained from the Culkin (1965) to salinity, the dissociation constant of boric acid from Dickson (1990) and the carbon dioxide solubility coefficient of Weiss (1974), with an estimated accuracy of ±5 $\mu mol/dm^3$. The pH and Talk were measured daily during the course of the experiment. NO_3^- and NH_4^+ were run on a Technicon autoanalyser. The detection limits of the analytic methods for nitrate and ammonium were 0.02 and 0.03 $\mu mol/dm^3$, respectively.

Dry mass was determined after experiment by drying at 65 °C until constant mass (48 h). The net growth rate (μ) of *Ulva pertusa* was calculated following the equation:

$$\mu = (\ln B_t - \ln B_0)t^{-1},$$

where B_t and B_0 are the biomasses (dry mass) of the algae at the end and the beginning of the experiment, respectively; and t is the number of days.

The flux (F) of CO_2 across the air-sea interface can be calculated from the relation:

$$F = ks[p_w(CO_2) - p_a(CO_2)], \qquad (1)$$

where k is the gas transfer velocity; s the solubility of CO_2 calculated after Weiss(1974); $p_w(CO_2)$ the partial pressure of CO_2 in the water; and $p_a(CO_2)$ the atmosphere partial pressure. The main factor influencing k is turbulence at the air-sea interface that is mainly generated by wind stress; thus, k is usually parameterized as a function of wind speed (Wanninkhof, 1992). We calculated F using the wind speed corrected to 10 m height. $p_a(CO_2)$ was assumed to be 36.07 Pa for the calculations of $\Delta p(CO_2)$.

3 Results

3.1 *Diurnal variation of pH in the water column*

There was a clear daily variation of pH, with maximum values at the end of incubation and minimum values at the beginning in both groups. The pH increased continuously with the time prolonged in each group (Fig. 1). In the NO_3^- addition groups, the pH varied from 8.298 to 8.684. Higher NO_3^- concentration resulted in a higher increase rate of pH when NO_3^- concentration was less than 71 μmol/dm^3 (Fig. 1). When the NO_3^- concentration was higher than 355 μmol/dm^3, the pH increase rate decreased. The pH of NO_3^- -3 group reached to 8.684 at the end and was 0.639 pH higher than the initial. In the NH_4^+ addition group, the pH had the same increasing tendency as NO_3^- addition group. The pH significantly increased with an increase of the NH_4^+ concentration from 4.97 to 49.7 μmol/dm^3. When the NH_4^+ concentration was more than 248.5 μmol/dm^3, the pH decreased. The pH of NH_4^+ -3 group increased faster than the other groups and had reached to 8.832 at the end. The pH in the N addition group increased faster than the control group, in which pH increased to 8.298 at the end and was 0.253 pH more than the initial.

3.2 *Influence of macronitrogen additions on dissolved inorganic carbon system*

There were significant differences in HCO_3^-, CO_3^{2-} and $p(CO_2)$ concentrations between the control group and N addition groups (Fig.2). Significant differences were also found among different levels of N addition groups. 71 μmol/dm^3 or 49.7 μmol/dm^3 NH_4^+ considerably decreased the concentration of HCO_3^-, whereas the other N levels did not greatly affect the concentration of HCO_3^- decreasing, though concentration of the other N addition groups decreased
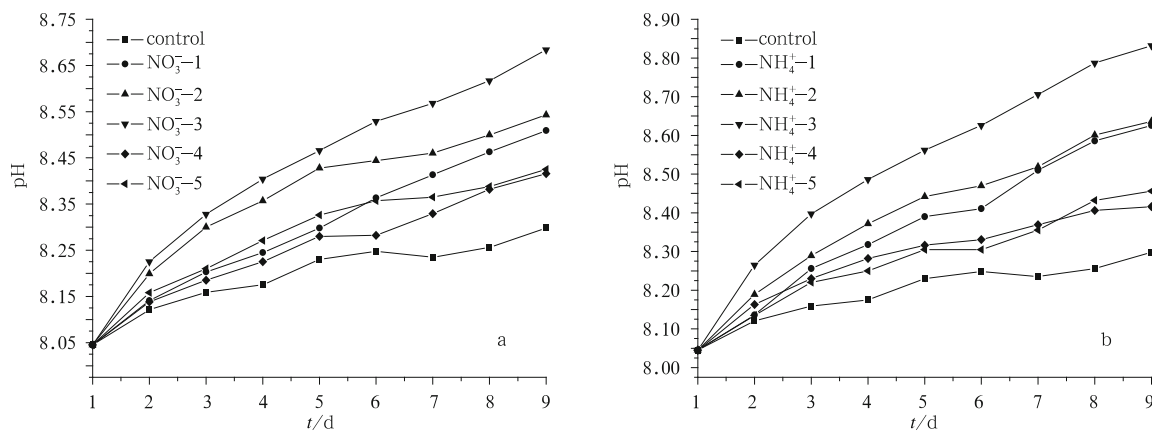

Fig. 1. Time courses of daily pH changes. ■7.1 μmol/dm^3 NO_3^- or 4.97 μmol/dm^3 NH_4^+ (background concentration), ●14.2 μmol/dm^3 NO_3^- or 9.94 μmol/dm^3 NH_4^+ addition, ▲35.5 μmol/dm^3 NO_3^- or 24.85 μmol/dm^3 NH_4^+ addition, ▼ 71 μmol/dm^3 NO_3^- or 49.7 μmol/dm^3 NH_4^+ addition, ♦ 355 μmol/dm^3 NO_3^- or 248.5 μmol/dm^3 NH_4^+ addition, ◄710 μmol/dm^3 NO_3^- or 497 μmol/dm^3 NH_4^+ addition.

greater than the control. For example, with NO_3^- in 71 μmol/dm^3 and NH_4^+ in 49.7 μmol/dm^3, the HCO_3^- concentration decreased 591 and 793 μmol/dm^3 at the end of incubation, respectively. While in the other N addition groups, the HCO_3^- concentration decreased only in the range of 295 to 531 μmol/dm^3. And the HCO_3^- concentration only decreased 162 μmol/dm^3 at the control group. Increasing NO_3^- concentration resulted in higher decreased extent of HCO_3^- when the

NO_3^- treatment level below 71 μmol/dm^3. When the NO_3^- concentration was higher than 355 μmol/dm^3, the decrease extent of HCO_3^- decreased. In the NH_4^+ addition groups, the decreased extent of HCO_3^- became higher when the NH_4^+ concentration was below 49.7 μmol/dm^3 and the decreased extent of HCO_3^- became lower when the NH_4^+ concentration was above 248.5 μmol/dm^3.
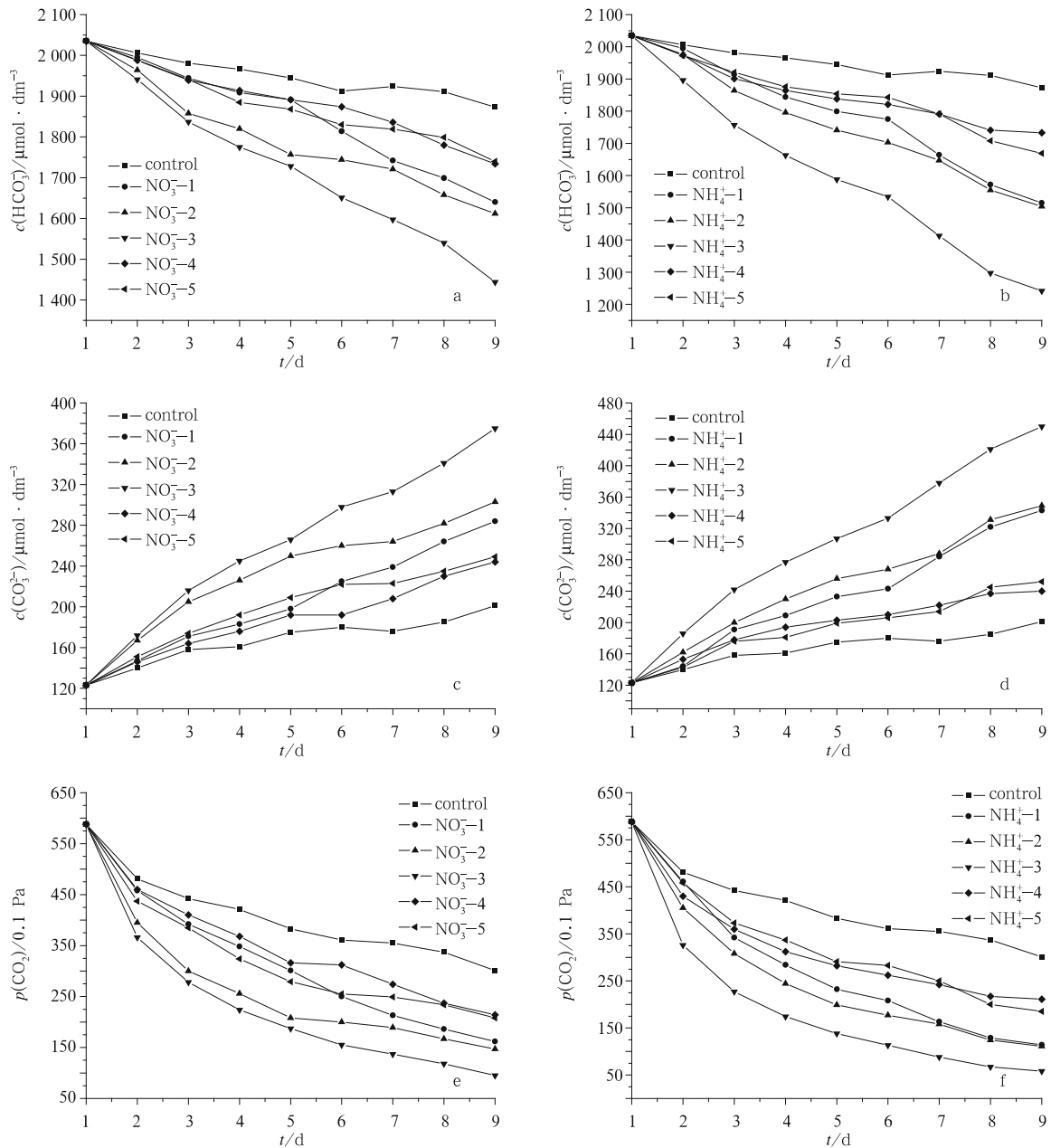

Fig. 2. The concentration changes HCO_3^-, CO_3^{2-} and $p(CO_2)$ with time. ■ 7.1 μmol/dm^3 NO_3^- or 4.97 μmol/dm^3 NH_4^+ (background concentration), ●14.2 μmol/dm^3 NO_3^- or 9.94 μmol/dm^3 NH_4^+ addition, ▲35.5 μmol/dm^3 NO_3^- or 24.85 μmol/dm^3 NH_4^+ addition, ▼71 μmol/dm^3 NO_3^- or 49.7 μmol/dm^3 NH_4^+ addition, ◆355 μmol/dm^3 NO_3^- or 248.5 μmol/dm^3 NH_4^+ addition, ◄710 μmol/dm^3 NO_3^- or 497 μmol/dm^3 NH_4^+ addition.

The CO_3^{2-} concentration significantly increased with an increase of the N concentration when the NO_3^- below 71 μmol/dm^3 and NH_4^+ below 49.7 μmol/dm^3 (Fig.2). At the end of the incubation, CO_3^{2-} concentration in NO_3^--3 group was three times higher than the initial and was the highest in all NO_3^- addition groups. In the NH_4^+ addition groups, the concentration of CO_3^{2-} increased with the time. The highest CO_3^{2-} concentration was in NH_4^+-3 group, which was 3.7 times higher than the initial. The lowest CO_3^{2-} concentration was in the groups of NH_4^+-4 and NH_4^+-5. The increasing extent of CO_3^{2-} in nutrient addition groups was all higher than the control group which increased 78 μmol/dm^3 in the end.

In NO_3^--3 and NH_4^+-3 groups, the $p(CO_2)$ decreased 50.0 and 83.7 Pa at the end of incubation, respectively (Fig.2). And both of them decreased more than the other N addition groups. The $p(CO_2)$ in the control group decreased less than the N addition groups, which only decreased 29.1 Pa at the end of the incubation. When the NO_3^- and NH_4^+ concentration were less than 71.0 and 49.7 μmol/dm^3, the decreasing extent of $p(CO_2)$ increased with the increasing N concentration. However, the decreasing extent of $p(CO_2)$ decreased when the concentration of NO_3^- and NH_4^+ was higher than 355.0 and 248.5 μmol/dm^3, respectively. At the end of incubation, the large variability of $p(CO_2)$ values ranged from 9.6 to 21.7 Pa in the NO_3^- addition groups and 5.9 to 21.4 Pa in the NH_4^+ addition groups.

3.3 CO$_2$ flux between the sea and the air

The variation of air-sea CO_2 flux was very obvious among different N addition groups (Fig.3). The result of air-sea CO_2 flux in each group shows that the seawater all changes from carbon source to carbon sink during the incubation period. Incubation groups with different forms and concentration of N have different air-sea CO_2 fluxes, which are all higher than the control group. The seawater changes from carbon source to carbon sink at different time. The air-sea CO_2 flux decreases from 116.3 mmol/(m^2·d) on the first day to –8.1 mmol/(m^2·d) on the last day in the control group, which changes from significant CO_2 source to slight CO_2 sink. In the NO_3^- addition groups, the maximum air-sea CO_2 flux is –97.5 mmol/(m^2·d) and the minimum is –45.9 mmol/(m^2·d). The concentration of NO_3^- at 71 μmol/dm^3 considerably increases the air-sea CO_2 flux, which is higher than the other NO_3^- addition levels. The 71 μmol/dm^3 group is also earlier than the other groups in changing from carbon source to carbon sink. In the NH_4^+ addition groups, the maximum air-sea CO_2 flux of the 49.7 μmol/dm^3 NH_4^+ group is –112.7 mmol/(m^2·d), which is higher than the other groups. The 49.7 μmol/dm^3 NH_4^+ group changes from CO_2 source to CO_2 sink earlier than the other NH_4^+ addition groups. The air-sea CO_2 fluxes of the NH_4^+-1 and NH_4^+-2 groups both are higher than the NH_4^+-4 and NH_4^+-5 groups at the end incubation. Compared with the two forms of N addition groups, the NH_4^+ addition groups are earlier than NO_3^- addition groups in changing from carbon source to carbon
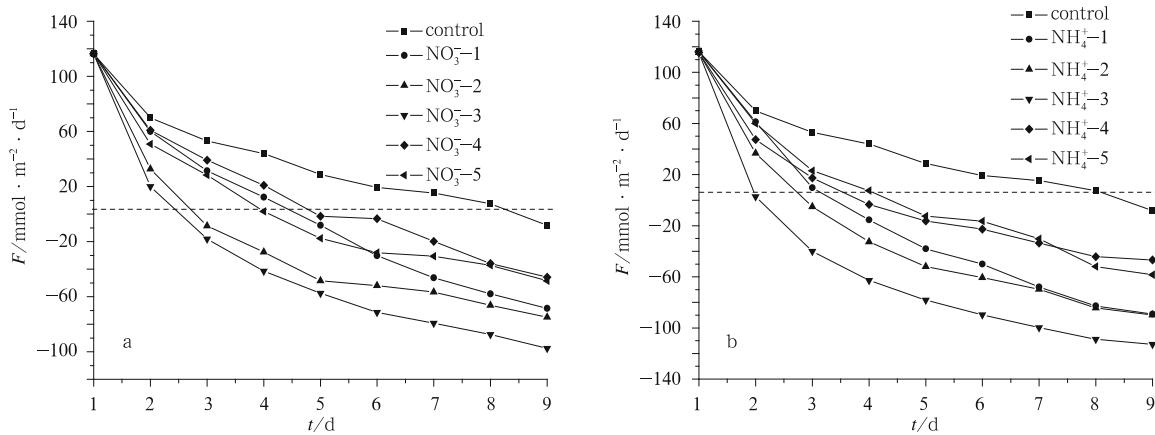
Fig. 3. Air-sea CO$_2$ fluxes in each box. Positive values sea-air fluxes, negative values air-sea fluxes. Both flux in mmol/(m^2·d). Positive values sea-air fluxes, negative values air-sea fluxes. Both flux in mmol/(m^2·d). ■7.1 μmol/dm^3 NO_3^- or 4.97 μmol/dm^3 NH_4^+ (background concentration), ● 14.2 μmol/dm^3 NO_3^- or 9.94 μmol/dm^3 NH_4^+ addition, ▲35.5 μmol/dm^3 NO_3^- or 24.85 μmol/dm^3 NH_4^+ addition, ▼71 μmol/dm^3 NO_3^- or 49.7 μmol/dm^3 NH_4^+ addition, ♦355 μmol/dm^3 NO_3^- or 248.5 μmol/dm^3 NH_4^+ addition, ◄710 μmol/dm^3 NO_3^- or 497 μmol/dm^3 NH_4^+ addition.

sink at the same N addition levels.

## 4 Discussion

4.1 *Change of components of inorganic carbon system during experiment*

Although pH is not used to directly quantify the concentration of a particular inorganic carbon species, pH is directly related to the equilibrium state of the CO_2 system. Therefore the change in seawater pH is the result of carbon cycling processes in the ocean. The previous studies have suggested that the pH of seawater is mainly governed by the concentration of total dissolved inorganic carbon and ionic equilibria between hydrogen ion and various inorganic carbon species (CO_2, HCO_3^- and CO_3^{2-}) in seawater (Carl and Chris, 2004; Zhang, 2000). Agneta et al. (2004) verified that the pH increasing was probably due to changes in DIC availability. However, the components of inorganic carbon system were mainly controlled by the metabolism of *Ulva pertusa* in this experiment. So the change in pH indicates an influence on seawater pH by *Ulva pertusa* activity (photosynthesis and respiration). When the photosynthesis rate was higher than the respiration rate and dissolved organic carbon decay rate, the ratio of $c(CO_2)$ to $c(CO_3^{2-})$ [$c(CO_2)$ was the concentration of CO_2] decreased, so pH increased. When the photosynthesis of *Ulva pertusa* was restricted, the respiration rate and dissolved organic carbon decay rate increased, the ratio of $c(CO_2)$ to $c(CO_3^{2-})$ decreased, so pH decreased (Menéndez et al., 2001). In this experiment, the $p(CO_2)$ decreased while CO_3^{2-} concentration increased all the time (Fig.2), the ratio of $c(CO_2)$ to $c(CO_3^{2-})$ decreased, the photosynthesis rate of *Ulva pertusa* was higher than its respiration rate, so pH increased all the time. However, from Fig.2 it can be seen that the increase rate of pH was faster than the rate of $p(CO_2)$ decreased, so there must be some other factors in contributing to pH increase. Zou (2005) demonstrated that green algae were capable of utilizing the reserve of HCO_3^- in seawater as its principal source to drive photosynthetic carbon fixation, which was closely associated with the extracellular dehydration of HCO_3^- mediated by the external carbonate alkalinity (CA). Therefore, the photosynthetic utilization of HCO_3^- in the light was involved in the catalyzed formation of CO_2 and hydroxide ion (OH^-) from HCO_3^- dehydration extracellularly. The formed CO_2 was transported across the plasmalemma and was ultimately fixed by Rubisco, while the formed

OH^- raised the pH value in the culture medium. As a consequence of that, the pH in the seawater rose fast.

In seawater, HCO_3^- is the main forms of DIC, about 85%, while CO_2 is only about 1% of the DIC pool, and CO_3^{2-} is less than 10% (Song et al., 2004). If CO_2 is removed, for example, due to photosynthetic uptake, new CO_2 is formed spontaneously from the ionic forms, but this process is slow when uncatalysed (Joachim and Bernd, 2004). In addition, the diffusion rates of all DIC forms are slow in water (Melissa et al., 2004). Thus, the growth of phytoplankton was limited when the CO_2 concentration decreased. In response to this situation, phytoplankton has developed several adaptive mechanisms which allow them to use the HCO_3^- pool. Margarita et al. (2001) suggested that there were two mechanisms for utilizing the bicarbonate by extracellular carbonic anhydrase. One mechanism operates via external dehydration of HCO_3^- followed by (passive or active) CO_2 entry through the plasma membrane. The second is the direct uptake of HCO_3^- through the plasma membrane. Extracellular carbonic anhydrase activity seems to be very common among macroalgae. It is apparently able to utilize CO_2 directly as well as HCO_3^- indirectly after dehydration to CO_2 by extracellular carbonic anhydrase (CA) (Marcel et al., 2002). *Ulva pertusa* also has the ability to use HCO_3^-, it can absorb HCO_3^- directly as carbon source of photosynthesis (Margarita et al., 2001). In this experiment, $p(CO_2)$ decreased rapidly at the beginning and decreased gradually at the end. It indicates that at the initial stages of experiment, CO_2 was enough for photosynthesis, but with the incubation time prolonged, the concentration of CO_2 became lower and lower. Stephen and Tom (2002) suggested that at a pH of 9 or greater, free CO_2 was almost completely unavailable. Thus, *Ulva pertusa* turned into absorbing HCO_3^- as the main carbon source for photosynthesis.

As shown in Fig. 2, the time course of CO_3^{2-} is similar to that of pH (Fig.1). CO_3^{2-} has the following equilibrium in water:

$$H^+ + CO_3^{2-} \rightleftharpoons HCO_3^-.$$
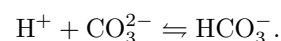
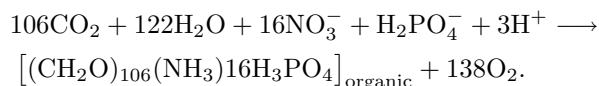Because CO_3^{2-} is unusable as a carbon source for photosynthesis (Marcel et al., 2002), the concentration change of CO_3^{2-} is governed by the concentration of H^+ and HCO_3^-. In this experiment, because CO_2 was not enough to maintain the photosynthesis of *Ulva pertusa*, HCO_3^- was used as a carbon source. While every HCO_3^- ion taken up by the *Ulva pertusa* one OH^-

was excreted to maintain electrical neutrality (Zou, 2005; Stephen and Tom, 2002). Then the equilibrium reaction of the inorganic carbon system was shifted toward the left when the concentration of H^+ decreased. Thus, the concentration of HCO_3^- decreased and the concentration of CO_3^{2-} increased (Fig.2).

4.2 Relationship of DIC with Ulva pertusa growth rate

The previous studies showed that the relationship between the changes of the inorganic carbonate system and nutrients during photosynthesis was frequently examined using the Redfield equation:

$$106CO_2 + 122H_2O + 16NO_3^- + H_2PO_4^- + 3H^+ \longrightarrow$$
$$\left[(CH_2O)_{106}(NH_3)16H_3PO_4\right]_{organic} + 138O_2.$$

Ferial et al. (2001) suggested that the drawdown of DIC and nutrients had fixed ratios. Yann et al. (2005) reported that the CO_2 coincided fairly well with the measured oxygen production. In this experiment we found that there was a significant negative-correlation between $\Delta c(DIC)$ and growth rate (μ) of *Ulva pertusa* ($r=-0.91$, $P < 0.000\ 1$, $n=11$) (Fig. 4). The results indicate that the decrease of DIC is mainly contributed to the growth of *Ulva pertusa* during the experiment. Similar findings were reported in the East China Sea (Tan et al., 2004). The changes in the carbonate system directly correspond to the growth rate of phytoplankton as indicated by the corresponding chlorophyll-a increase.
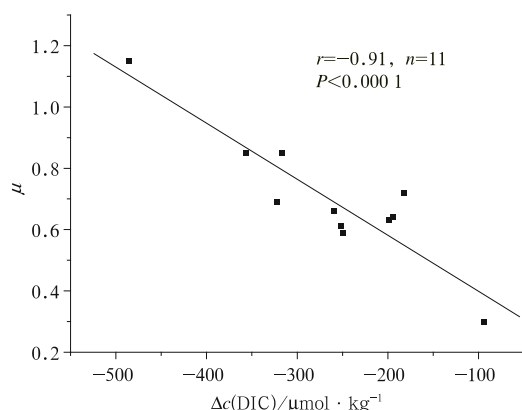

Fig. 4. Correlation between Δc (DIC) and growth rate (μ) of *Ulva pertusa*.

4.3 Influence of macro-nitrogen additions on DIC transfer

Significant decrease of DIC concentration was observed during the incubation (Fig.5). Compared with

the control group, the decrease of DIC concentration varied from -74 to -151 $\mu mol/dm^3$ in the NO_3^- addition groups and from -109 to -232 $\mu mol/dm^3$ in the NH_4^+ addition groups, respectively. From Fig. 4, it can be seen that at the same N addition levels, the decreasing extent of DIC concentration in the NO_3^- addition groups is lower than the NH_4^+ addition groups. That is because the *Ulva pertusa* assimilates NH_4^+ easier than NO_3^-. The previous research suggests that the algae needed six ferredoxins in NO_3^- reduction which consumed four times energy than NH_4^+ (Buyukates and Roelke, 2005). Dong and Zhao (2006) found that the algae incubated with NH_4^+ were six times than that incubated with NO_3^- in absorbing inorganic carbon. Thus, NH_4^+ can significantly induce *Ulva pertusa* in absorbing DIC.
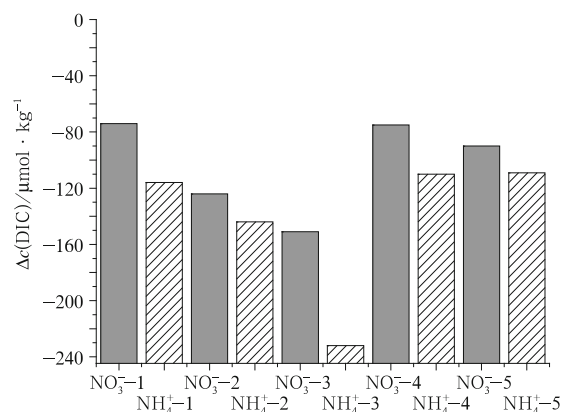

Fig. 5. Final DIC concentration in the groups, expressed as decrease of DIC concentration compared with the control.

In N addition groups, the concentration of DIC decreased first and then increased with the increasing of N addition levels (Fig. 5). In the NO_3^- addition groups, the concentration of DIC decreased from -74 $\mu mol/dm^3$ in the NO_3^-–1 group to -151 $\mu mol/dm^3$ in the NO_3^-–3 group. In the NH_4^+ addition groups, the concentration of DIC decreased from -116 $\mu mol/dm^3$ in the NH_4^+–1 group to -232 $\mu mol/dm^3$ in the NH_4^+–3 group. Thus, there must be a critical concentration of NO_3^- and NH_4^+ with *Ulva pertusa*. When the NH_3^- and NH_4^+ concentration was lower than the critical concentration, it would accelerate the photosynthesis in absorbing DIC. Contrarily, it would restrain the photosynthesis of *Ulva pertusa* when the NO_3^- and NH_4^+ concentration was higher than the critical concentration. Kevin (2005) reported that N lack or excessive both would significantly restrain the photosynthesis in

absorbing DIC. For the NH_4^+ addition groups, there exists a thermodynamic equilibrium:

$$NH_4^+ \rightleftharpoons NH_3 + H^+.$$

NH_3 is toxic to cell because it can permeate cellular membranes freely and then restrict the cell growth (Li et al., 2006). NH_3 concentration increases with the increasing of NH_4^+ concentration. When the NH_4^+ was more than a certain concentration, it might restrain the growth of algae. The previous studies suggest that under different environment factors, such as temperature, salinity, concentration of CO_2, irradiance and pH, etc., the variety of photosynthesis and nutrient assimilation was significantly (Xia and Huang, 2010; Miao and Wu, 2002). Katwijk et al. (1997) tested possible toxic effects of high N concentration in the water layer on the *Zostera marina* and found a stronger toxic effect at 20°C than at 15°C. Ge and Fang (2006) revealed that NH_4^+ was toxic to *Ulva pertusa* at concentration above 70 μmol/dm^3 under lower irradiance of 106 lx. During this experiment, it would facilitate the growth of *Ulva pertusa* and accelerate the uptake of DIC when the concentration of NH_4^+ below 49.7 μmol/dm^3. Whereas, it would restrain the growth of *Ulva pertusa* when the concentration of NH_4^+ was above 248.5 μmol/dm^3. The phenomena can also be seen with that the variety (Δm) of dry mass of *Ulva pertusa* in the end experiment (Table 2).

Table 2. Growth parameters of *Ulva pertusa*

	m/g	Δm/g	$mu(\%)$
Control	3.603	0.082	2.33
NO_3^--1	3.696	0.175	4.97
NO_3^--2	3.719	0.198	5.62
NO_3^--3	3.782	0.261	7.41
NO_3^--4	3.712	0.191	5.42
NO_3^--5	3.737	0.216	6.13
NH_4^+-1	3.783	0.262	7.44
NH_4^+-2	3.728	0.207	5.88
NH_4^+-3	3.891	0.370	10.50
NH_4^+-4	3.708	0.187	5.31
NH_4^+-5	3.702	0.181	5.14

5   Conclusions

This study demonstrated that macronitrogen (NO_3^- and NH_4^+) considerably changed pH, species concentration of inorganic carbon system and air-sea CO_2 flux in a controlled laboratory experiment. Higher N concentrations generally all facilitate the biological uptake of HCO_3^- and CO_2 and promote the

pH and CO_3^{2-} concentration. DIC decreased significantly with a middle N concentration of 71 μmol/dm^3 NO_3^- or 49.7 μmol/dm^3 NH_4^+. There was a significant negative-correlation between Δc(DIC) and growth rate of *Ulva pertusa* which indicated that the decrease of DIC was mainly contributed to the growth of *Ulva pertusa* during the experiment. At the same N addition levels, the decreasing extent of DIC concentration of the NO_3^- addition groups was lower than the NH_4^+ addition groups. Proper N addition can promote the growth rate of *Ulva pertusa*. However, excessive N addition became toxic for *Ulva pertusa*. The air-sea CO_2 fluxes obtained demonstrate that the macronitrogen addition increases the net CO_2 uptake from the atmosphere.

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