Carbon Dynamics in Aquatic Ecosystems in Response to Elevated Atmospheric CO_2 and Altered Nutrients Availability

By

Chao Song

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Chairperson: Ford Ballantyne IV

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Sharon Billings

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Joy Ward

Date Defended: April 18, 2011

The Dissertation Committee for Chao Song

certifies that this is the approved version of the following dissertation:

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Abstract

Aquatic ecosystems will experience altered inorganic carbon, nitrogen and phosphorous availability in the future due to elevated atmospheric CO_2, stronger stratification and anthropogenic activities. Despite its importance in modulating global carbon cycles, how carbon dynamics in aquatic ecosystem response to the future global change remains largely unknown. Here we performed a chemostat experiment to study how equilibrium carbon dynamics response to elevated CO_2 and altered N, P availability. Our results show that elevated CO_2 led to enhanced photosynthetic carbon uptake and DOC production. DOC occupied larger percentage in total organic carbon production in high CO_2 environment. N addition stimulated biomass carbon accumulation. Collectively, in the future, high CO_2 and low nutrient availability lead to high C: nutrient ratio in both biomass and dissolved organic carbon. It indicates a possible change in nutrient limitation and increase in recalcitrant dissolved organic carbon as long term carbon sequestration. Total carbon consumption remains unclear and will depend on the net effects of depleted nutrients and elevated CO_2.

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Introduction

Aquatic ecosystems play a crucial role in modulating the global carbon cycle. Phytoplankton in the ocean fix 48.5 petagrams of carbon per year, contributing almost half of the global net primary production (Caldeira & Wickett, 2003). Large carbon fixation capacity and carbon pool size make aquatic ecosystems important potential carbon sink for anthropogenic carbon emission. It is estimated that the oceanic carbon sink accounts for 48% of the total fossil-fuel and cement-manufacturing carbon emissions from 1800 to 1994 (Sabine *et al.*, 2004). Small changes in carbon fixation or transport, for example, caused by biological response to the changing environment in aquatic ecosystems, would have strong impacts on global carbon dynamics.

Two main processes, the biological carbon pump and the microbial carbon pump, are involved in carbon storage in aquatic ecosystems. The biological pump is a process in which carbon fixed in the upper layer by primary producers is transported to deep water as particulate organic matter or dissolved organic matter (Eppley & Peterson, 1979). A small fraction of particulate matter sinks down to the sediments and is stored for long time. This long term carbon storage is of primary concern as it provides a potential sink for human induced carbon emission (Ducklow *et al.*, 2001). However, dissolved organic matter produced by primary producers can be taken up by bacterial and archae. While most of the carbon consumed by bacteria and archae is respired and released as CO_2, fraction of fixed carbon is released via direct exudation, virallysis of microbial cells or particulate matter degradation as recalcitrant dissolved organic matter (Jiao *et al.*, 2010). Radiocarbon evidence show that the age of recalcitrant dissolved carbon is about 4000-6000 years (Bauer *et al.*, 1992). This microbially mediated production of recalcitrant dissolved organic carbon is known as the microbial carbon pump. The current inventory of recalcitrant

dissolved carbon pool is 624 petagrams (Hansell *et al.*, 2009), which is comparable to the atmospheric carbon pool (Falkowski *et al.*, 2000).

Future climate changes are likely to affect carbon dynamics in aquatic ecosystems in two ways. First, rising atmospheric CO_2 will increase the inorganic carbon availability in aquatic ecosystems. Atmospheric CO_2 can physically dissolve in water or react with water according to:

$$CO_2(\text{atmospheric}) \Leftrightarrow CO_2(\text{dissolved})$$

$$CO_2 + H_2O \Leftrightarrow H^+ + HCO_3^-$$

$$HCO_3^- \Leftrightarrow H^+ + CO_3^{2-}$$

Higher partial pressure of CO_2 will result in more dissolved CO_2 and more total inorganic carbon in water. Simultaneously, pH will decrease and the carbon chemistry will be shifted to more bicarbonate and less carbonate. Assuming a "business as usual" scenario, by the end of next century, dissolved CO_2 concentration in ocean will reach as high as 30 $\mu mol\ kg^{-1}$, which is three times as high as the current concentration (Houghton, 1996). Elevated atmospheric CO_2 concentration and the associated increase in inorganic carbon in aquatic ecosystem will influence C availability for primary producers in two ways. Some phytoplankton species can directly take up dissolved CO_2 into the cell whereas other species have carbon concentrating mechanism, in which phytoplankton cells catalyze the conversion from HCO_3^- to CO_2 with carbonic anhydrase and utilize CO_2 as the carbon source (Elzenga *et al.*, 2000). As both dissolved CO_2 and bicarbonate concentration will increase with elevated atmospheric CO_2, carbon availability increases and therefore carbon fixation by primary producers is likely to be enhanced. Evidence from elevated CO_2 perturbation experiment with natural phytoplankton communities in mesocosms shows a significant increase in total inorganic carbon consumption. Higher total carbon consumption is related to build up of dissolved organic carbon in the surface layer as well

as loss of organic matter in the surface layer through sinking (Riebesell *et al.*, 2007). As a result, aquatic ecosystems might be able to buffer increases in atmospheric CO_2, potentially sequestering additional carbon. Second, future global climate change and anthropogenic activities can change the nutrient regime in aquatic ecosystems and therefore affect carbon dynamics indirectly. Global warming will strengthen stratification in lakes and ocean, which suppresses vertical mixing across density gradient and reduces the nutrient influx from nutrient-enriched deep water to surface layer (Sarmiento *et al.*, 2004; Peeters *et al.*, 2007). The end result, therefore, is a decrease in nutrient availability for phytoplankton growth. On the other hand, human activities greatly increase nutrient flow into rivers, lakes, and costal marine ecosystems, which may offset the effects of greater stratification to some extent (Howarth *et al.*, 2011).

Altered nutrient availability has the potential to affect phytoplankton physiology and therefore influences carbon dynamics in aquatic ecosystems. A global analysis shows that freshwater and marine ecosystems are often limited by N and P. Simultaneously adding N and P will stimulate primary production in aquatic ecosystems (Elser *et al.*, 2007). In addition, interaction between elevated CO_2 and nutrient concentration can change the stoichiometry of phytoplankton cells and organic matter they produce, potentially affecting long term carbon sequestration (Hutchins *et al.*, 2007). However, studies on how interactions between high CO_2 and nutrient concentration influence carbon dynamics in aquatic ecosystems are rare.

Despites great progresses in understanding carbon dynamics in aquatic ecosystems, several important questions are still unclear. 1) We lack understanding of equilibrium carbon dynamics under continuous elevated CO_2 and altered nutrient inflow. Most carbon perturbation

experiments supply a pulse CO_2 and nutrients and observe phytoplankton or ecosystem response. The experimental systems experience rapid depletion of inorganic carbon and nutrient, and therefore are not in equilibrium with the environment. Steady state response of aquatic ecosystems to rising CO_2 and altered nutrient availability may provide insights into long term carbon dynamics in future climate change scenarios. 2) The interactive effects of nutrient availability and high CO_2 on aquatic ecosystem carbon dynamics are not well studied. Carbon dynamics are closely related to nutrient availability, especially the most limiting nutrient, typically N or P. Knowledge of how nutrient availability mediates carbon uptake will contribute to a more previous understanding of how carbon dynamics are affected in the future. To address these questions, we grew naturally occurring freshwater phytoplankton and bacterial assemblages in semi-continuous culture under continuously elevated CO_2 with altered N: P input stoichiometry.

Methods and Materials

Phytoplankton culture

We sampled natural phytoplankton and bacterial communities from the Frank B. Cross Reservoir (39.05^0N, 95.18^0W, May 28^{th}, 2010) at the University of Kansas Field Station to perform the experiment. We took a 10 liter integrated sample of the epilimnion (0-6 m) to obtain the most diverse and representative assemblage of natural occurring phytoplankton and bacterial. In the lab, we filtered sample water through a 50 μm mesh filter to eliminate zooplankton. We inoculated each experimental culture with 2 ml of filtered water sample in 148 ml WC growth medium (Guillard *et al.*, 1975). Inorganic nitrogen (as NaNO3) and phosphorus (as KH2PO4) were added to the growth medium to obtain two nitrogen concentrations (40 μmol L^{-1} and 160 μmol L^{-1}) and two phosphorus concentrations (4 μmol L^{-1} and 16 μmol L^{-1}), yielding 4 nutrient

combinations. We grew experimental cultures in 250 ml baffled polycarbonate flasks with vented screw cap to enable gas exchange. We placed culture flasks in two growth chambers (Conviron BDR16, Winnipeg, Canada) with a constant temperature of 20 ^{0}C and a constant light intensity of 100 μmol m^{-2} s^{-1}. We set the CO_2 concentration in one chambers at 380 ppm and at 700 ppm in the other. Cultures were exposed to constant CO_2 concentration during the course of experiment. In all, we had two nitrogen levels, two phosphorus levels and two CO_2 levels, yielding eight N-P-CO_2 combinations. We replicated each N-P-CO_2 treatment three times, giving us 24 total experimental cultures.

We grew cultures in semi-continuous culture, diluting daily and shaking flasks 2-3 times per day to provide some degree of suspension and mixing. We pipetted out 5 ml of well-mixed culture and replaced the volume with 5 ml growth medium every day to maintain a semi-constant nutrient inflow rate of 1/30 d^{-1}. We measured optical density (OD) at 480 nm daily and used OD readings as an indicator for biomass. We assumed cultures were at steady state once mean daily OD was stationary.

Measurements

We harvested cultures once they reached steady state and made the following measurements: pH, cholorphyll a, dissolved organic carbon, nitrate, total dissolved nitrogen, phosphate and total dissolved phosphorus. Upon harvest, 50 ml sample from each flask was filtered through a Whatman GF/F glass fiber filter. Filter papers were air dried in dark for 12 hours and processed immediately for Chlorophyll a analysis. Chlorophyll a concentration was measured by spectrophotometric methods after 90% hot ethanol extraction in the dark for 24 hours (Sartory & Grobbelaar, 1984). We estimated biomass carbon from Chlorophyll a concentration with a

conversion factor of 100 g C g Chl^{-1} (Behrenfeld *et al.*, 2005). Filtrate was used to determine nitrate, total dissolved nitrogen, phosphate, total dissolved phosphorous and dissolved organic carbon concentration. We measured dissolved organic carbon by high temperature combustion with a Shimadzu TOC-5000A carbon analyzer. Nitrate analysis was accomplished by reduction of nitrate to nitrite by nitrate reductase, and reaction of the resulting nitrite with Griess reagents (Campbell *et al.*, 2006). Phosphate was measured based on malachite green-molybdate binding reaction (Van Veldhoven & Mannaerts, 1987). We measured total dissolved nitrogen and total dissolved phosphorus by oxidizing the filtrate with persulfate (Langner & Hendrix, 1982) and measure the resulting nitrate and phosphate with the same methods.

With the exception of one flask, in all cultures we analyzed, we did not find significant densities of nitrogen fixers. Therefore, we assumed that all availability nitrogen and phosphorous in the experimental cultures came from growth medium. Therefore, we assumed that total N concentration and total P concentration were equal to the inorganic N and P concentration in the growth medium. Based on this assumption, we calculated following N and P pool concentration.

Dissolved organic nitrogen = Total dissolved nitrogen – Nitrate.

Biomass nitrogen = Total nitrogen – Total dissolved nitrogen

Dissolved organic phosphorous = Total dissolved phosphorous – Phosphate

Biomass phosphorous = Total phosphorous – Total dissolved phosphorous

Inorganic carbon concentration was calculated based on chemical equilibrium, temperature and pH. The chemical equilibria and equilibrium constants are:

$$CO_{2\ (gas)} \Leftrightarrow CO_{2\ (water)} \quad K_{CO2}$$

$$CO_{2\ (water)} + H_2O \Leftrightarrow H_2CO_3 \quad K_a$$

$$H_2CO_3 \Leftrightarrow H^+ + HCO_3^- \quad K_1$$

$$HCO_3^- \Leftrightarrow H^+ + CO_3^{2-} \quad K_2$$

The value of each equilibrium constant at 20 ^{0}C was calculated based on standard value at 25 ^{0}C (Lide, 2003) and the Van't Hoff equation. Therefore, concentration of different inorganic carbon form can be calculated as:

$$[CO_2]_{(water)} = K_{CO2} \times [CO_2]_{(gas)}$$

$$[H_2CO_3] = K_a \times [CO_2]_{(water)}$$

$$[HCO_3^-] = K_1 \times [H_2CO_3]/[H^+]$$

$$[CO_3^{2-}] = K_2 \times [HCO_3^-]/[H^+]$$

Theoretical calculations

We were able to calculate net photosynthetic carbon uptake rate and organic carbon production rate based on the steady state culture of the experimental cultures. Carbon dynamics in each flask can be modeled as fluxes between several carbon pools (Figure 1). The main carbon pools are dissolved inorganic carbon (DIC), dissolved organic carbon (DOC) and particulate carbon (BioC). Because the culture is in artificial growth medium, we assume all particulate carbon is biomass carbon from phytoplankton. For the DIC pool, diffusion of atmospheric CO_2 into the culture was the carbon sources. Net photosynthetic carbon uptake was the process that removed carbon from DIC pools. Growth medium and experimental culture were both in equilibrium with atmospheric CO_2 in the same environment and they contained same amount of DIC. Therefore, dilution did not contribute to net change in DIC pool. For the biomass carbon pool, carbon enters via photosynthesis as inorganic carbon is fixed from dissolved inorganic carbon while respiration returns part of the fixed carbon to DOC pool. The death, excretion or growth of phytoplankton may release organic carbon to water. We refer this process as DOC

production. For DOC pool, DOC produced by phytoplankton was the only source and daily dilution was the only mean for carbon removal. The carbon dynamics can be described by the following differential equations:

$$d([DIC])/dt = \text{atmosphere-water diffusion} + \text{respiration} - \text{photosynthesis}$$

$$d([DOC])/dt = \text{DOC production} - \text{dilution}$$

$$d([BioC])/dt = \text{photosynthesis} - \text{respiration} - \text{growth, excretion, death} - \text{dilution}$$

Because we fixed the dilution rate and held it constant, carbon removal rate from each pool is 1/30 of the pool size per day. At steady state, which we assumed once OD values became stationary, we can set the above differential equations to 0 and calculate total atmosphere-water diffusion, net photosynthetic carbon uptake rate (photosynthesis- respiration) and organic carbon production rate at steady state. All flux rates are expressed as changes in concentration per day.

$$\text{Total atmosphere-water } CO_2 \text{ diffusion rate} = ([DOC]+[BioC])/30$$

$$\text{Net photosynthetic carbon uptake rate} = ([DOC]+[BioC])/30$$

$$\text{DOC production rate} = [DOC]/30$$

Statistical analysis

We performed multi-way analysis of variance analyze the effects of CO_2, nitrogen levels and phosphorus levels on different carbon pool size (DIC, DOC and BioC) and flux rate. We compared C: Nutrient ratio in biomass and dissolved organic matter by paired t-test. All statistical analyses were performed in R package (version 2.12.1).

Results

Culture growth

Most cultures reached a steady state, as defined by stationary OD, after 38 days of dilution (Figure 2). Only one culture with 40 μmol L^{-1} N, 16 μmol L^{-1} P and 380 ppm CO_2 treatment still kept growing at the time of harvest. Species examination showed that this culture was dominated by nitrogen fixation cyanobacteria (*Anabaena sp*.). Since this culture was not at its steady state at the time of harvest, we excluded it from further analysis.

Carbon pool size

Dissolved CO_2 concentrations, as calculated under the assumption of equilibrium with the atmosphere, was higher in high CO_2 treatment (Table 1) as dissolved CO_2 concentration was proportional to the partial pressure of CO_2 in the air. The calculated concentration of dissolved CO_2 was 14.82 μmol L^{-1} and 27.30 μmol L^{-1} under 380 ppm and 700 ppm CO2 respectively. The pH of cultures exposed to 380 ppm CO_2 was 7.29 on average and 7.20 on average for those experiencing 700 ppm CO_2. Within CO_2 treatment, pH was consistent. Bicarbonate was the main form of DIC in both CO_2 treatments, occupying 89.7% (380 ppm CO2) and 87.6% (700 ppm CO2) of total DIC. We calculated total DIC concentration under 380 ppm and 700 ppm CO_2 to be 143.55 μmol L^{-1} and 220.10 μmol L^{-1} respectively (Table 1). As we calculated the inorganic carbon concentration based on chemical equilibrium, nutrient availability had no effects on inorganic carbon pool size.

Total organic carbon (TOC) is the sum of DOC and BioC. TOC concentration was significantly higher at high CO_2 (Figure 3a,Table 2, $p=0.017$) and N treatment (Figure 3a, Table 2, $p=0.005$). BioC, calculated from chlorophyll a concentration and the conversion factor of 100 g C/g Chl, showed strong positive response to nitrogen addition (Figure 3b, Table 2, $p<0.001$). BioC was consistently lower under high CO_2 treatment but the effects were not statistically significant at

5% significance level (p=0.1005). High CO_2 had significant effects on DOC concentration. DOC concentration was significantly higher under 700 ppm CO_2 treatment (Figure 3c, Table 2, p=0.002).

Carbon flux rate

High N and high CO_2 treatment significantly stimulated net photosynthetic carbon uptake rate, which equals the TOC production rate at steady state (Figure 4a). Within P and CO_2 treatments, increases in N concentration from 40 μmol L^{-1} to 160 μmol L^{-1} increased TOC production rate by 20%-235%. Within N and P treatment, increase in atmospheric CO_2 increased photosynthetic carbon uptake rate by 8%-203%, with the minimum increase found in low nutrient treatment (40 μmol L^{-1} N and 4 μmol L^{-1} P). Although the analysis of variance did not show significant effects of CO_2:N, CO_2:P and CO_2:N:P interaction on photosynthetic carbon uptake, the general trend was that the increase in photosynthetic carbon uptake was more pronounced when nutrient availability was high.

The rate of DOC production was significantly higher under high CO_2 treatment (Figure 4b). Within N and P treatment, increase in CO_2 leads to 27% to 1000% increase in DOC production rate. However, nutrient availability and C:nutrient interaction did not have significant effects on DOC production rate. The proportion of DOC in TOC production was highly variable, ranging from 6.7% to 92.3%. High CO_2 and low N led to a high percentage of DOC in TOC production (Figure 4c). This was consistent with the fact that DOC production was higher under high CO_2 treatment and that BioC was lower under low N treatment.

Nutrient and C:nutrient stoichiometry

Most input inorganic nitrogen and phosphate were taken up by phytoplankton and bacteria. Nitrate and phosphate concentration at steady state was 1-10% and 0.4-16% of input nitrate and phosphate concentration respectively. None of the treatment resulted in significant difference on nitrate and phosphate concentration (Figure 5a, b). Dissolved organic nitrogen was significantly lower under high CO_2 ($p=0.035$) and high P ($p=0.009$) treatments. However, dissolved organic nitrogen concentration was constrained between 20-30 $\mu mol\ L^{-1}$ and did not vary much across treatment (Figure 5c). Dissolved organic phosphorous was uniformly low across treatments and did not response significantly to any treatment. Its concentration ranged from 0.06-1.29 $\mu mol\ L^{-1}$ and was 0.5-16.7% of total phosphorous concentration (Figure 5d). However, input nutrient concentration strongly influenced biomass nitrogen and phosphorous. Biomass nitrogen was significantly higher under high N ($p<0.001$) and P ($p=0.01$) treatment (Figure 5e). Biomass nitrogen concentration was highly correlated with input nitrate concentration (correlation coefficient>0.99) since total dissolved nitrogen (nitrate+ dissolved organic nitrogen) ranged only between 21.6-31.3 $\mu mol\ L^{-1}$ and did not vary much across N treatment. Biomass phosphorous showed similar pattern (Figure 5f). Across all treatment, biomass P accounted for more than 75% to total phosphorous and was highly correlated to input P (correlation coefficient >0.99).

The molar ratio of biomass C:N ranged from 0.88 to 18.69. Biomass C:N ratio was significantly lower under high CO_2 (Figure 6a, Table 4, $p=0.019$) and high N treatment (Figure 6a, Table 4, $p<0.001$). C:N ratio in dissolved organic matter had a different response to high CO_2 and was significantly higher under high CO_2 treatment (Figure 6c, Table 4, $p=0.005$). Compared to biomass C:N, N was more depleted in dissolved organic matter and therefore, C:N in dissolved organic matter was significantly higher (paired t-test, $p=0.007$). Biomass C:P ratio was significantly higher under high N (Figure 6b, Table 4, $p=0.001$) and low P (Figure 6b, Table 4,

p=0.001) treatment. Nitrogen had more pronounced effects on biomass C:P ratio when P level was low, evidenced by significant effects of N:P interaction (Figure 6b, Table 4, p=0.005). C:P ratio in dissolved organic matter was generally higher under high CO_2 treatment (Figure 6d, Table 4, p=0.009) and the effects was more significant at high P level (Figure 6d, Table 4, p=0.029).

Discussion

Enhanced net primary production in high CO_2 environment

Our experiment showed that at steady state, continuously elevated atmospheric CO_2 significantly enhanced net photosynthetic carbon uptake rate. Net primary production under high CO_2 treatment increased significantly and consistently across different nutrient availability (Figure 4a). Traditionally, inorganic carbon was not considered as a limiting factor for primary production in aquatic ecosystems (Raven & Johnston, 1991; Falkowski, 1994). The concentration of HCO_3^- in aquatic ecosystems is ~200 μmol L^{-1} and dissolved CO2 is ~10 μmol L^{-1} at equilibrium with the atmosphere (25^0C). Most carboxylation enzymes in marine phytoplankton require 25-35 μmol L^{-1} CO_2 to saturate (Raven & Johnston, 1991). However, HCO_3^- cannot be directly used for carbon fixation and has to be converted to CO_2 inside or outside the cell by carbonic anhydrase. This poses potential carbon limitation for phytoplankton species that cannot concentrate CO_2 internally by actively transporting and converting HCO_3^- (Riebesell *et al.*, 1993). Studies have shown that carboxylation of coccolithophorids *Emiliania huxleyi* (Engel, 2005) and *Gephyrocapsa Oceanica* (Riebesell *et al.*, 2000) were far below saturation at present atmospheric CO_2 concentration. For species that have carbon concentrating mechanism, elevated CO_2 could still stimulate carbon fixation because carbon concentrating process is usually limited by available energy (Riebesell, 2004) or enzyme cofactor of carbonic

anhydrase, such as zinc (Morel *et al.*, 1994). Carbonate chemistry predicts that in our experiment, dissolved CO_2 should increase from 14.82 μmol L^{-1} at 380 ppm atmospheric CO_2 to 27.30 μmol L^{-1} at 700 ppm atmospheric CO_2. The significant increase in dissolved CO_2 may have provided enough increase in the concentration of dissolved CO2 to stimulated primary production.

Our results are consistent with the enhanced net primary production in elevated atmospheric CO_2 found in other studies, but differ importantly in that we report a continuous enhancement under steady stat condition. For example, a relative recent CO_2 perturbation experiments with marine phytoplankton community in large mescosoms showed a 39% increase in total carbon consumption at1050 ppm CO_2 (Riebesell *et al.*, 2007). And during incubations of natural phytoplankton assemblages from a nutrient poor central Atlantic transect, enhanced carbon fixation of up to 15% in response to three-times increased CO_2 was observed (Hein & Sand-Jensen, 1997). The concordant results from these experiments suggest a common response of aquatic ecosystems to pulse CO_2 increase. In our experiment, we observed enhanced net primary production at steady state. Combined with results from previous study, out data suggest that enhancement in total carbon consumption in response to elevated atmospheric CO_2 is likely to be maintained if atmospheric CO_2 remains high. Increased total organic carbon pool size in aquatic ecosystems has the potential to sequester additional carbon and buffer increasing atmospheric CO_2.

Altered carbon distribution pattern in response to high CO_2

Total organic carbon production was significantly higher under high CO_2 treatment (Figure 3a). This was primarily driven by significantly enhanced DOC production (Figure 3c). In contrast,

biomass carbon was consistently lower at 700 ppm CO_2 (Figure 3b) although the response was not statistically significant (p=0.1005). Therefore, the percentage of DOC in TOC production significantly increased in high CO_2 environment (Figure 4c). A similar pattern was also observed in a CO_2 perturbation experiment, in which accumulation of dissolved organic matter accounts for the enhanced inorganic carbon consumption (Riebesell *et al.*, 2007).

This altered carbon distribution pattern is likely to have implication for long term carbon storage in aquatic ecosystems because DOC plays an important role in sinking biogenic particle and recalcitrant dissolved organic carbon formation. A recent study showed that Polysaccharide aggregation or transparent exopolymetric particle (TEP) formed from dissolved organic matter is an important pathway to convert dissolved into particulate organic matter and mediates vertical carbon flux (Engel *et al.*, 2004). Enhanced TEP production in a high CO_2 environment was evident in both natural phytoplankton community (Engel, 2002) and in monospecific cultures of the diatom *Thalassiosira weissflogii* and the coccolithophorid *Emiliania huxleyi (*Engel, 2005). In addition, dissolved organic carbon is subject to microbial consumption. Recalcitrant dissolved organic carbon can be formed from labile carbon and remain in the upper layers of quatic ecosystems due to microbial activities. In a 36 day incubation, *Pseudomonas chlororaphis* depleted the sole carbon source, d-glucose, within 2 days and 5-10% of carbon derived from d-glucose persisted until the end of the experiment (Biddanda & Benner, 1997). A year long incubation of marine bacterial assemblages show that up to 50% of carbon derived from labile carbon source persisted until the end of the incubation, indicating that bacterial can generate relative stable carbon from labile sources efficiently (Ogawa *et al.*, 2001). The successive and repetitive processing of dissolved organic matter by bacteria transforms the reactive carbon to recalcitrant forms and builds up a long term carbon reservoir (Jiao *et al.*, 2010). Since dissolved

organic carbon can be transformed to recalcitrant form by various pathways, enhancement in total organic carbon fixation and shif in the distribution of TOC towards more DOC production creates the potential for a larger and more stable carbon reservoir in aquatic ecosystem.

Stoichiometric analysis demonstrated that C:N:P stoichiometry in recalcitrant dissolved organic matter (~3511:202:1) was different from that in labile dissolved organic matter (199:20:1) and particulate organic matter (106:16:1) (Hopkinson & Vallino, 2005). In our experiment, the C:N ratio of dissolved organic matter increased significantly in the high CO_2 treatments (Figure 6c). The mean C:N ratio of dissolved organic matter increased from 6.34 at 380 ppm CO_2 to 21.99 at 700 ppm CO_2. Dramatic increases in C:N ratio in dissolved organic matter suggested a possible shift from labile dissolved carbon to recalcitrant dissolved organic carbon and indicates an overall increase in dissolved organic carbon stability in response to high CO_2. However, more research is needed to identify the chemical composition and evaluate the stability of increasing DOC pools found in our experiment.

Nutrient effects and C:nutrient stoichiometry

N and P are considered to be limiting nutrients in aquatic ecosystems (Sterner & Elser, 2002). N and P addition generally leads to a significant response in biomass accumulation across various aquatic ecosystems (Schindler, 1977; Elser *et al.*, 2007). The results from our experiment were partly consistent with this general pattern. Varying input P concentration did not significantly influence carbon dynamics (Figure 3, 4). N addition stimulated biomass carbon accumulation (Figure 3b) but did not have significant effects on dissolved organic carbon (Figure 3c). At steady state, inorganic N, P and total dissolved N, P was consistently low across all the treatments. Most N and P was incorporated into biomass, evidenced by a high correlation

between biomass N, P and input N, P. As a result, C: nutrient ratio in biomass was driven mainly by nutrient availability while C:nutrient ratio in dissolved organic matter was influenced by CO_2 (Figure 6). High P availability will result in lower C:P ratio in biomass. High N availability will simultaneously stimulated biomass accumulation and N uptake. The net effect of higher N availability is lower C:N ratio in biomass. As a consequence, we would expect biomass C: nutrient ratios to shift upward in response to more pronounced stratification (and therefore lower nutrient availability) in aquatic ecosystem. Significant increases in C: nutrient may cause an elemental imbalance between phytoplankton and zooplankton and affect nutrient recycling efficiency. Relatively more carbon than nutrients will be released from zooplankton in response to increased C: nutrient ratios in phytoplankton. Reduced nutrient recycling may potentially feedback on phytoplankton biomass C:nutrient ratio, resulting in an additional shift up in C:nutrient ratio in phytoplankton biomass (Hessen & Anderson, 2008). Therefore, climate change induced decreases in nutrient availability are likely to increase the C: nutrient ratio in aquatic ecosystems.

Conclusions

The experimental results described here provided insights into equilibrium response of aquatic ecosystems to altered CO_2 and nutrient availability. We observed a significant increase in total carbon fixation, an altered carbon distribution pattern towards more DOC and significantly lower C:N ratio in dissolved organic matter in high CO2 environments. Increased N availability led to greater biomass accumulation and lower biomass C:N stoichiometry. Under predicted future global climate change, aquatic ecosystems will experience stronger stratification (and therefore less nutrient supply from deep water), and elevated atmospheric CO_2. If we can extrapolate the results of this experiment, aquatic ecosystems are likely to have less biomass accumulation, more

dissolved organic carbon production and higher C:N ratio in both biomass and dissolved organic matter. More dissolved organic carbon with higher C:N ratio will potentially function as a mean for stable carbon sequestration. But the total carbon fixation will depend on the extend of nutrient drawdown and atmospheric CO_2 increase.

Tables and figures

Table 1 Mean concentration of dissolved inorganic carbon (DIC), dissolved CO_2, biomass carbon (BioC) and dissolved organic carbon (DOC) in each N, P and CO_2 treatment. Units: µmol L-1.

Nutrient	CO2	DIC	Dissolved CO2	BioC	DOC
160 µM N	380	143.55	14.82	252.65	188.60
16 µM P	700	220.10	27.30	150.23	693.16
40 µM N	380	143.55	14.82	98.33	33.42
16 µM P	700	220.10	27.30	60.48	339.44
160 µM N	380	143.55	14.82	365.03	205.88
4 µM P	700	220.10	27.30	307.29	534.70
40 µM N	380	143.55	14.82	106.185	311.23
4 µM P	700	220.10	27.30	55.61	397.70

Table 2 Summary results of analysis of variance testing whether CO2, N, P and their interaction has effects on total organic carbon (TOC), biomass carbon (BioC) and dissolved organic carbon (DOC) concentration.

	Factor	D.f	Sum of square	F	P-value
TOC	CO2	1	56791515	7.16	0.017
	N	1	83957954	10.57	0.005
	P	1	9481818	1.19	0.29
	CO2:N	1	9407586	1.19	0.29
	CO2:P	1	5360679	0.68	0.42
	N:P	1	3623595	0.46	0.51
	CO2:N:P	1	1206831	0.15	0.70
	Residuals	16	126969184		
BioC	CO2	1	3338052	3.04	0.11
	N	1	30751821	27.99	<0.001
	P	1	4007810	3.65	0.074
	CO2:N	1	278133	0.25	0.62
	CO2:P	1	55066	0.05	0.83
	N:P	1	3834722	3.49	0.08
	CO2:N:P	1	177869	0.16	0.69
	Residuals	16	17578871		
DOC	CO2	1	87666670	13.63	0.002
	N	1	13085762	2.03	0.17
	P	1	1160585	0.18	0.68
	CO2:N	1	12920877	2.01	0.18
	CO2:P	1	6502373	1.01	0.32
	N:P	1	14913644	2.32	0.15
	CO2:N:P	1	458077	0.07	0.79
	Residuals	16	102918721		

Table 3 Summary results of analysis of variable testing whether photosynthetic carbon uptake rate, dissolved organic carbon production rate and percentage of dissolved organic carbon production in total carbon production response to CO2, N and P treatment.

	Factor	D.f	Sum of square	F	P-value
Photosynthetic carbon uptake Rate	CO2	1	63102	7.16	0.017
	N	1	93287	10.58	0.005
	P	1	10535	1.19	0.29
	CO2:N	1	10453	1.19	0.29
	CO2:P	1	5956	0.68	0.42
	N:P	1	4026	0.46	0.51
	CO2:N:P	1	1341	0.15	0.70
	Residuals	16	141077		
Dissolved organic carbon production rate	CO2	1	97407	13.63	0.002
	N	1	14540	2.03	0.17
	P	1	1290	0.18	0.68
	CO2:N	1	14357	2.01	0.18
	CO2:P	1	7225	1.01	0.32
	N:P	1	16571	2.32	0.15
	CO2:N:P	1	509	0.07	0.79
	Residuals	16	114354		
Percentage of dissolved organic carbon production in total carbon production	CO2	1	0.7883	38.56	<0.001
	N	1	0.13219	6.46	0.022
	P	1	0.03951	1.93	0.18
	CO2:N	1	0.00018	0.0087	0.93
	CO2:P	1	0.12963	6.34	0.023
	N:P	1	0.20097	9.82	0.0064
	CO2:N:P	1	0.04018	1.96	0.18
	Residuals	16	0.32734		

Table 4 Results of analysis of variance examining whether CO2, N and P has treatment effects on C:nutrient stoichiometry in biomass and dissolved organic matter.

	Factor	D.f	Sum of square	F	P-value
Biomass C/N	CO2	1	72.40	6.83	0.019
	N	1	236.88	22.35	<0.001
	P	1	3.58	0.33	0.57
	CO2:N	1	48.70	4.60	0.048
	CO2:P	1	2.26	0.21	0.65
	N:P	1	0.75	0.07	0.79
	CO2:N:P	1	3.75	0.35	0.56
	Residuals	16	169.57		
Biomass C/P	CO2	1	728.1	1.00	0.33
	N	1	11446.8	15.77	0.001
	P	1	19058.4	26.25	0.001
	CO2:N	1	10.1	0.014	0.91
	CO2:P	1	245.8	0.34	0.57
	N:P	1	7616.5	10.49	0.005
	CO2:N:P	1	3.4	0.005	0.95
	Residuals	16	11614.5		
Dissolved organic matter C/N	CO2	1	1263.84	10.54	0.005
	N	1	237.38	1.98	0.18
	P	1	10.93	0.091	0.77
	CO2:N	1	202.31	1.69	0.21
	CO2:P	1	143.79	1.20	0.29
	N:P	1	420.92	3.51	0.08
	CO2:N:P	1	27.52	0.23	0.64
	Residuals	16	1918.40		
Dissolved organic matter C/P	CO2	1	4519211	8.82	0.009
	N	1	663025	1.29	0.27
	P	1	25151	0.049	0.83
	CO2:N	1	2572	0.005	0.94
	CO2:P	1	2928943	5.72	0.029
	N:P	1	17280	0.034	0.86
	CO2:N:P	1	2636369	5.15	0.038
	Residuals	16	8197704		

Figure 1 Diagram for carbon dynamics in the experimental chemostat.

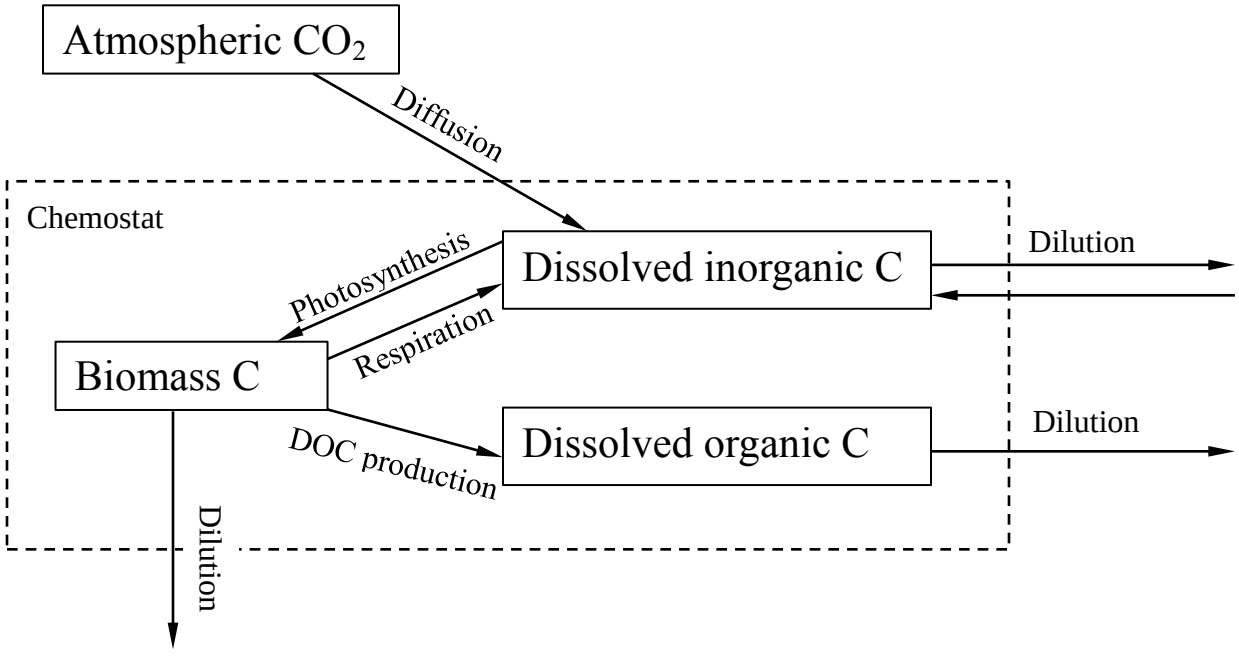


Figure 2 Optical density at 480nm. Each panel shows OD trajectory within a specific CO2, N and P treatment.

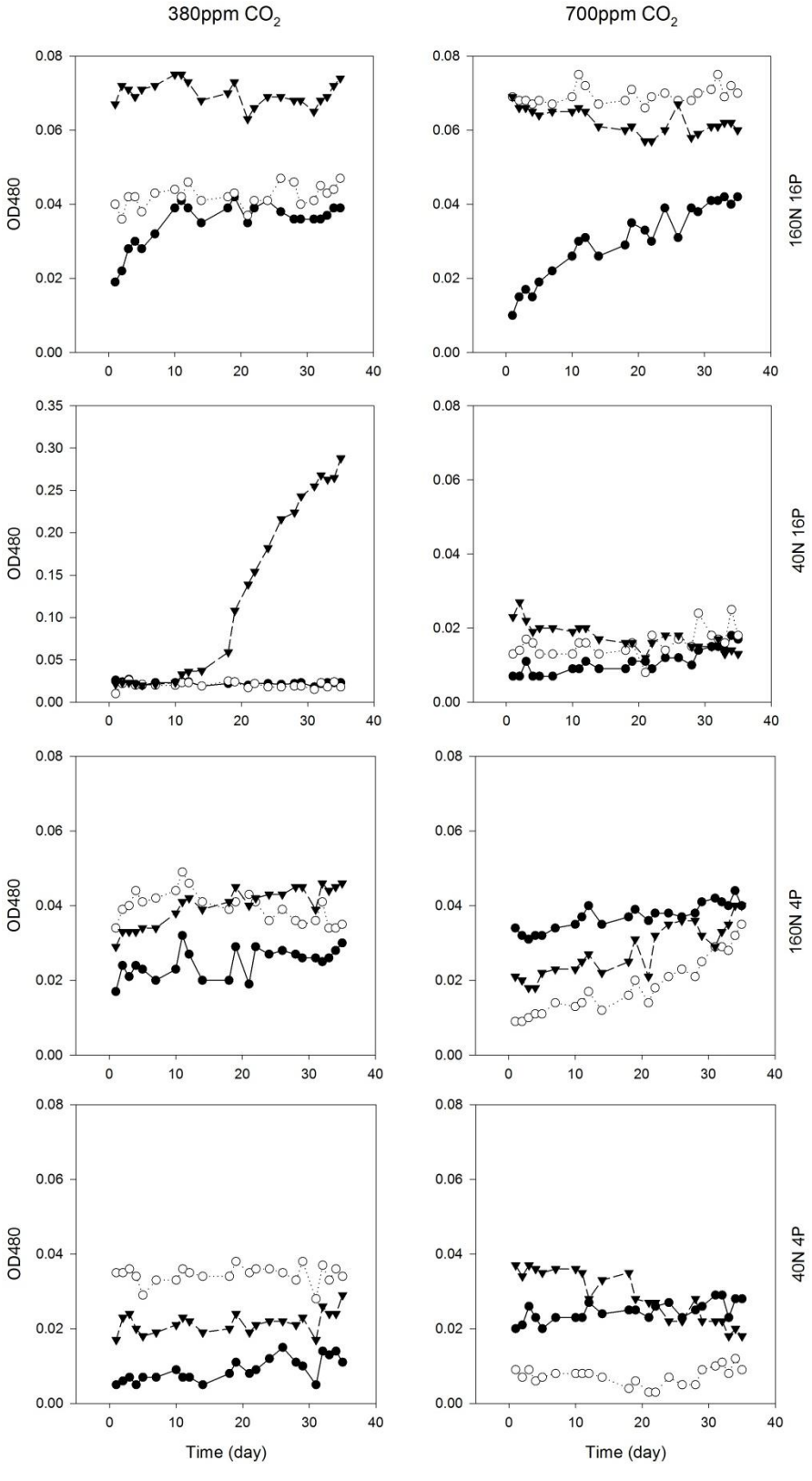


Figure 3 Carbon pool size at steady state. a) total organic carbon concentration, b) biomass carbon concentration, c) dissolved organic carbon concentration. Error bar shows the standard deviation.

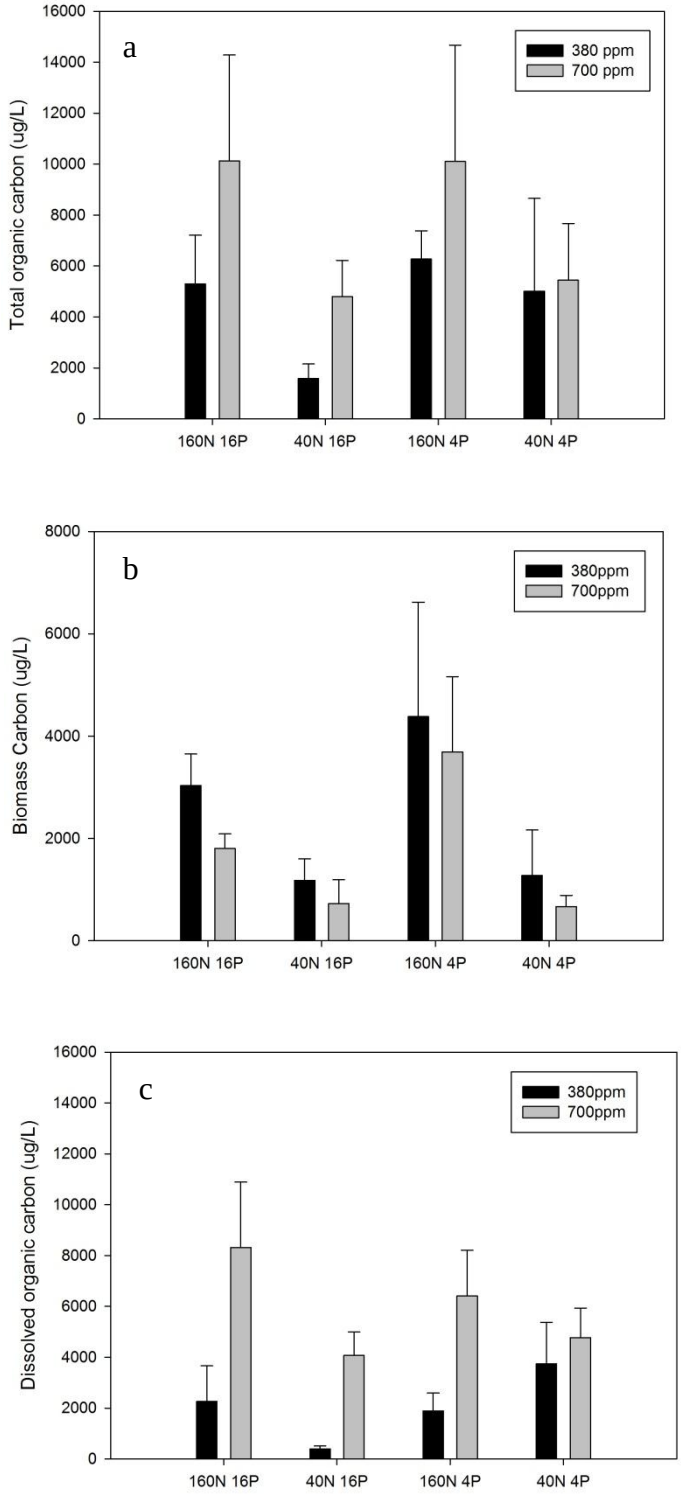


Figure 4 Carbon flux rate. a) photosynthetic carbon uptake rate, b) dissolved organic carbon production rate, c) percentage of dissolved organic carbon production in total organic carbon production. Error bar shows the standard deviation.

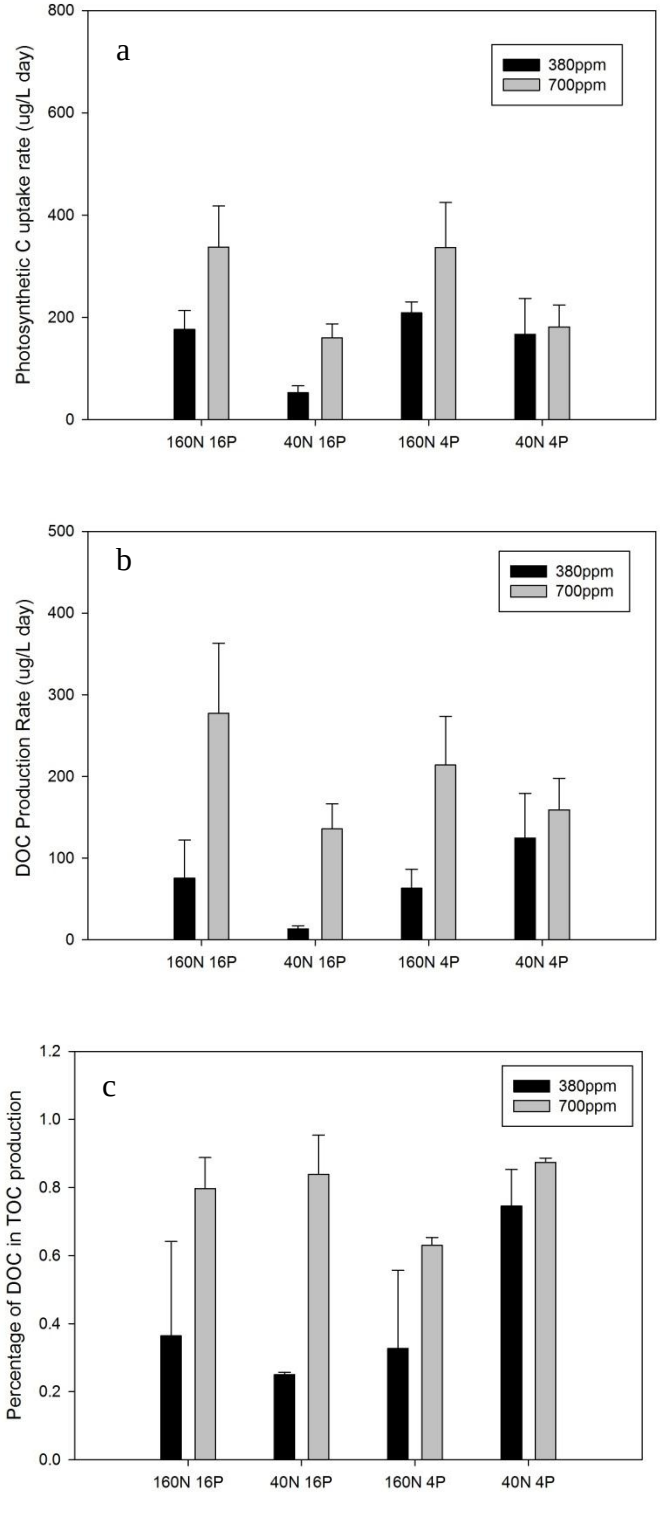


Figure 5 Nutrients concentration at steady state. a) nitrate concentration, b)phosphate concentration, c) dissolved organic N concentration, d) dissolved organic P concentration, e) biomass N concentration, f) biomass P concentration. Error bar shows the standard deviation.

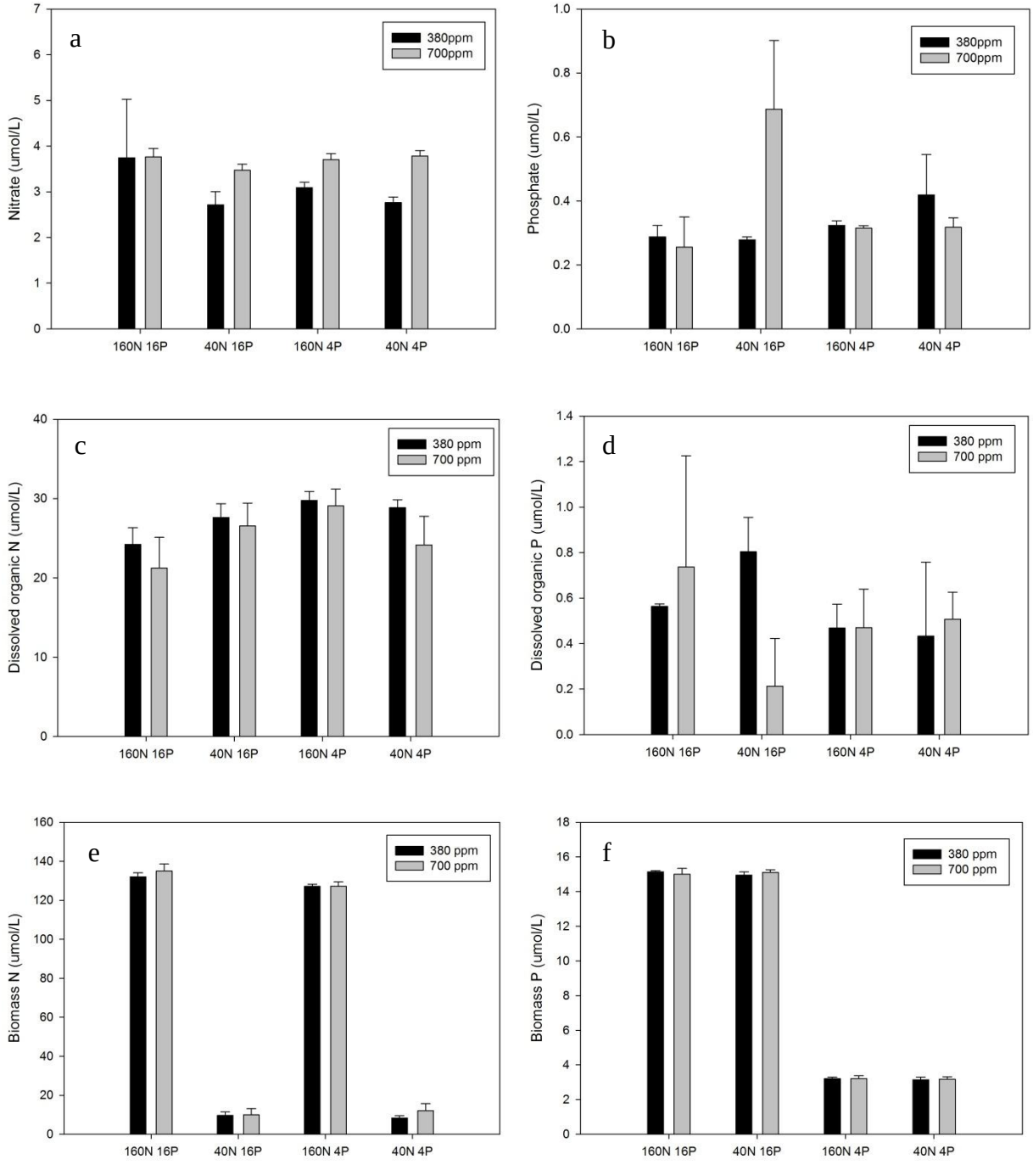
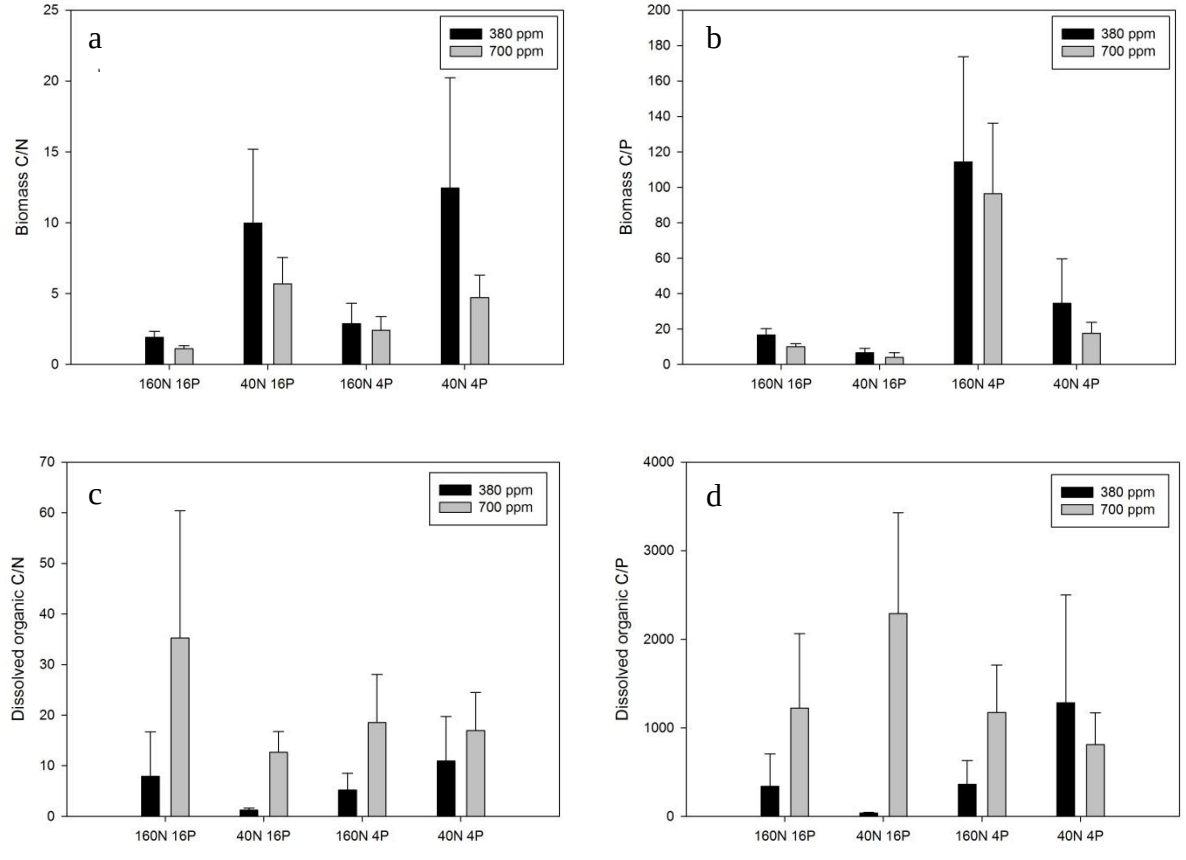


Figure 6 C:nutrient stoichiometry in biomass and dissolved organic matter. a) C:N ratio in biomass, b) C:P ratio in biomass, c) C:N ratio in dissolved organic matter, d) C:P ratio in dissolved organic matter. Error bar shows the standard deviation.



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