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Enhanced dissolved organic carbon production in aquatic ecosystems in response to elevated atmospheric CO_2

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Abstract Although aquatic ecosystems are a major carbon reservoir, how their carbon dynamics will respond to increasing concentrations of atmospheric CO_2 is not well understood. The availability of essential nutrients has the potential to modify carbon fluxes under elevated CO_2 by altering carbon processing and storage in the biota. Here, we describe a semi-continuous culture experiment with natural phytoplankton and bacteria assemblages designed to investigate (1) how carbon dynamics in aquatic ecosystems respond to continuously elevated atmospheric CO_2, and (2) whether carbon fluxes resulting from elevated CO_2 are modified by changes in inorganic nitrogen and phosphorus availability. Our results showed that elevated CO_2 led to significant increases in photosynthetic carbon uptake, despite a decrease in the algal chlorophyll *a* concentrations and no significant change in total algal biovolume. This enhancement of inorganic carbon uptake was accompanied by a significant increase in dissolved organic carbon (DOC) production. Concurrent increases in the C/N and C/P ratios of dissolved organic matter also suggested that DOC stability increased. Nutrient availability, especially nitrogen availability, had strong effects on inorganic carbon uptake and biomass carbon pools. In contrast, CO_2-enhanced DOC production was not significantly affected by varying concentrations of inorganic nitrogen and phosphorus. Our study underscores the importance of DOC as a potential carbon sink in aquatic ecosystems. The observed responses to changes in CO_2 and nutrient availability could have important implications for long-term carbon cycling in aquatic ecosystems, and highlight the potential buffering capacity of aquatic ecosystems to future environmental change.

Keywords Aquatic ecosystems · Carbon · Dissolved organic carbon · Elevated CO_2 · Nutrient availability

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Introduction

Aquatic ecosystems play a crucial role in modulating the global carbon (C) cycle. For example, phytoplankton fix 48.3 petagrams of C per year, accounting for almost half of global net annual primary production (Geider et al. 2001). The oceans alone harbor 38,000 petagrams C, and are responsible for modulating

atmospheric CO_2 concentrations over geologic timescales (Schlesinger 1997). Over recent millennia, the oceans have played an important role in modulating the global flux of anthropogenically-produced C. Sabine et al. (2004) have estimated that the oceanic C sink accounted for 48 % of the total fossil-fuel and cement-manufacturing C emissions between 1800 and 1994. In addition, inland freshwaters are estimated to receive 1.9 petagrams C from a combination of background and anthropogenic sources, 1.0 petagrams of which are either exchanged with atmosphere or buried in sediments (Cole et al. 2007). By virtue of the vast size of their C reservoirs, the world's aquatic ecosystems are important C sinks for anthropogenically-produced CO_2, and even small changes in C fixation or transport in aquatic ecosystems can potentially have strong impacts on global C dynamics.

Biological processes play a multifaceted role in governing C flux and storage in aquatic ecosystems. The biological pump, which refers to the transport of photosynthetically fixed C to the deep ocean and subsequent sinking to the sediments as particulate matter (Eppley and Peterson 1979), has been of primary interest because it provides a potential sink for anthropogenic CO_2 emission (Ducklow et al. 2001). More recently, the production of recalcitrant dissolved organic carbon (DOC) by bacteria through direct exudation, viral lysis of microbial cells, or particulate matter degradation has been highlighted as another potential C sequestration mechanism (Jiao et al. 2010). Laboratory incubations have shown that DOC derived from labile C sources can persist over long experimental timeframes (Ogawa et al. 2001), and radiocarbon studies have revealed that the age of recalcitrant DOC in the oceans is about 4,000–6,000 years (Bauer et al. 1992). The enormous amount of recalcitrant DOC in the ocean (Hansell et al. 2009), combined with its slow turnover rate, makes it particularly important to understand how the fluxes into and out of this refractory C pool will be influenced by increased C availability from rising atmospheric CO_2.

A major consequence of rising atmospheric CO_2 concentrations is an increase in inorganic C in aquatic ecosystems. As CO_2 dissolves into and reacts with water, concentrations of carbonic acid, bicarbonate, and carbonate come to an equilibrium determined by the partial pressures of CO_2 in the atmosphere and in the water. As the partial pressure of CO_2 in the atmosphere increases due to anthropogenic activities, the carbonic acid equilibrium shifts, increasing both dissolved CO_2 and bicarbonate concentrations. This shift could potentially stimulate photosynthetic C fixation, and evidence from mesocosm experiments performed under elevated CO_2 (Riebesell et al. 2007) and from large scale oceanic inorganic C surveys (Hein and Sand-Jensen 1997) both suggest that aquatic ecosystems act as potential C sinks.

Although C availability undoubtedly influences rates of C fixation, the response of phytoplankton to rising atmospheric CO_2 may be confounded by changing nutrient availability in aquatic ecosystems. Despite the fact that nitrogen (N) and phosphorus (P) often limit primary production in aquatic ecosystems and are very tightly linked to phytoplankton growth (Schindler 1977; Smith 1979, 2006; Elser et al. 2007; Harpole et al. 2011), the addition of N can potentially suppress photosynthetic rates, presumably via competition for metabolites between the Calvin–Benson cycle and N assimilation pathways (Elrifi and Turpin 1986). This means that uptake of inorganic C by phytoplankton in response to elevated CO_2 is likely to be mediated by the availability of inorganic N, and also potentially by any essential resources (Hopkinson et al. 2010). Phytoplankton also have the ability to regulate C concentrating mechanisms to maintain their cellular C/N stoichiometry (Falkowski and Raven 2007), implying a potential shift in C and N acquisition economics under elevated CO_2, and resulting in different patterns of C and N flux through aquatic ecosystems. For example, inorganic C and nutrient availability can change the stoichiometry of phytoplankton cells and organic matter they produce, thereby influencing nutrient recycling and organic matter recalcitrance (Ballantyne et al. 2008, Hessen 2008; van de Waal et al. 2010). Changes in DOC stoichiometry in turn can potentially affect long term C sequestration (Hutchins et al. 2007). Considering that warming increases the intensity of thermal stratification of lakes and oceans, which in turn reduces vertical mixing and nutrient flux from nutrient-rich deep water into the euphotic zone (Sarmiento et al. 2004; Peeters et al. 2007), it is imperative to study the effects of increasing CO_2 in the context of varying nutrient availability.

Despite great progress in understanding C dynamics in aquatic ecosystems, we still lack a comprehensive understanding of equilibrium C dynamics under conditions of continuous elevated CO_2 and altered nutrient

inflow. Most C perturbation experiments to date have supplied a pulse of CO_2 and nutrients, and then monitored any subsequent phytoplankton or ecosystem responses. Such experimental systems experience transient dynamics that involve the rapid depletion of inorganic C, N, and P, and are therefore not at equilibrium. Studying the steady-state responses of aquatic ecosystems to rising CO_2 and altered nutrient availability may provide additional new insights into long term C dynamics under future climate change scenarios. In this study, we conducted a semi-continuous culture experiment with natural freshwater phytoplankton and bacterial assemblages (1) to explore how elevated atmospheric CO_2 influences C dynamics in aquatic ecosystems at steady state, and (2) to assess the extent to which the effects elevated CO_2 may be modified by inorganic N and P availability.

Materials and methods

Phytoplankton culture

We used natural phytoplankton and bacterial communities obtained from the Frank B. Cross Reservoir (39.05°N, 95.18°W, May 28th, 2010) at the University of Kansas Field Station to inoculate our laboratory experiments. Although we used freshwater phytoplankton and bacterial assemblages, the findings from our experiment should be generalizable to marine ecosystems because previous studies have reported strong similarities in nutrient uptake and growth, resource competition, productivity (Halterman and Toetz 1984; Kilham and Hecky 1988; Smith 2006), inorganic C uptake, and photosynthetic apparatus (Falkowski and Raven 2007) between freshwater and marine phytoplankton. After taking a 10 L integrated sample of the euphotic zone (0–6 m) to obtain a diverse and representative assemblage of natural occurring phytoplankton and bacteria, we filtered the water through a 50 μm mesh filter to eliminate macrozooplankton grazers. We then inoculated 24 250 mL baffled polycarbonate flasks with 2 ml of the filtered lakewater sample and 148 mL WC growth medium. Each culture flask was fitted with a vented screw cap to allow full gas exchange. We modified the WC medium (Guillard 1975) by omitting $NaHCO_3$. Inorganic N ($NaNO_3$) and P (KH_2PO_4) were adjusted in the WC medium to obtain two N concentrations (40 and 160 μmol L^{-1}) and two P concentrations (4 and 16 μmol L^{-1}), yielding 4 nutrient combinations. We chose these N and P concentrations based on a limnological survey of multiple reservoirs and lakes in this region, and thus they represent the natural range of nutrient availability experienced by the phytoplankton and bacteria community used as the inoculum in this experiment. Each of the 4 nutrient treatments was replicated 3 times at two atmospheric CO_2 concentrations for a total of 24 flasks. We placed these culture flasks in 2 Conviron BDR16 growth chambers at a constant temperature of 20 °C and light intensity of 100 μmol photons m^{-2} s^{-1}. One growth chamber was maintained at 380 ppm CO_2 and the other was maintained at 700 ppm CO_2. Cultures were exposed to constant CO_2 concentration during the course of experiment to mimic a constant increase in atmospheric CO_2. We grew the natural phytoplankton and bacteria assemblage in semi-continuous culture, diluting daily (pipetting out 5 mL of well-mixed culture and replacing the volume with 5 mL growth medium every day to maintain a semi-constant nutrient inflow rate of 1/30 day^{-1}) and shaking flasks manually 2–3 times per day to provide cell resuspension and mixing. Each day, we measured the optical density (OD) at 480 nm of a water sample from the 5 mL outflow of each flask using a spectrophotometer as a turbidometric measure of total algal biomass. We assumed that the cultures had attained steady state once the mean daily OD was stationary.

Measurements

We harvested the cultures once they reached steady state, and made the following measurements: pH prior to harvest, cell size and abundance of each phytoplankton genus, chlorophyll *a*, DOC, nitrate plus nitrite, total dissolved N, dissolved inorganic phosphate, and total dissolved P. Upon harvest, a 50 mL aliquot from each flask was filtered through a Whatman GF/F glass fiber filter. Filters were air-dried in the dark for 12 h, and then processed immediately for chlorophyll *a* analysis. Chlorophyll *a* concentrations were measured using spectrophotometric methods after 90 % hot ethanol extraction of dried filters in the dark for 24 h (Sartory and Grobbelaar 1984). We measured the cell size and abundance of each phytoplankton genus presented in the sample with an ocular micrometer using a compound microscope under 400×

magnification. Cell volume of each phytoplankton genus was calculated based on a geometric approximation of its cell shape (Sun and Liu 2003). We then estimated phytoplankton biomass C from total cell volume using the following equations established from empirical data: Pg C $cell^{-1} = 0.216 \times biovolume^{0.939}$ for non-diatom species, and Pg C $cell^{-1} = 0.228 \times biovolume^{0.811}$ for diatoms (Menden-Deuer and Lessard 2000). Bacterial biomass C was estimated indirectly using the empirical equation log(bacterial biomass C) = 0.219 × log(phytoplankton biomass C) + 1.39, which was reported in a meta-analysis of limnetic studies by Simon et al. (1992). We computed total biomass C (BioC) as the sum of phytoplankton biomass C and bacterial biomass C, and used BioC as an estimate of particulate C because detritus was observed to be minimal to nonexistent in our experimental cultures.

We used the GF/F filtrate to determine nitrate plus nitrite, total dissolved N, phosphate, total dissolved P, and DOC concentrations. DOC was measured by high temperature combustion with a Shimadzu TOC-5000A carbon analyzer. We measured concentrations of nitrate and nitrite using a nitrate reductase-based reduction step and the standard Griess reaction (Campbell et al. 2006). We measured phosphate using a malachite green-molybdate binding reaction (Van Veldhoven and Mannaerts 1987; Biovision K410-500 phosphate colorimetric assay kit). Total dissolved N and total dissolved P were measured by first oxidizing the filtrate with persulfate (Langner and Hendrix 1982), and then using the same analytical methods employed for nitrate and phosphate.

With the exception of one flask, in all cultures that we analyzed, we did not find significant densities of N_2-fixing cyanobacteria. Therefore, we assumed that nitrogen fixation was minimal to non-existent, and all N and P in the experimental cultures was derived from the WC medium. As a consequence, we assumed that total N and total P concentrations in the flasks were equal to the inorganic N and P concentration in the inflowing growth medium. Based on this assumption, we calculated biomass N and P as the difference between total N and P and our measured values for total dissolved N and P. Similarly, dissolved organic N and P were calculated as the difference between total dissolved N and P and measured concentrations of inorganic N (nitrate + nitrite) and P (phosphate). We did not account for ammonia in our calculation, but ammonia is unlikely to be significant under the highly oxidizing conditions associated with a dense phytoplankton cultures grown in well-lit conditions. In addition, the absence of macrozooplankton eliminated the possibility of ammonification from excreted organic N. Furthermore, any ammonia that was produced through mineralization of organic matter was presumably utilized as fast or faster than nitrate, considering the high N demand in our experimental cultures as evidenced by consistently low steady-state nitrate and nitrite concentrations.

Calculations

We modeled C dynamics in each flask as fluxes between three primary C pools: dissolved inorganic C (DIC), DOC, and BioC (Fig. 1b). We defined net DOC production rate as DOC production rate minus DOC uptake rate, and we defined net C uptake rate as photosynthetic rate minus respiration rate. Employing these two definitions, we focused our modeling on the DOC and BioC pool dynamics in our experimental cultures using the following two differential equations:

$$\frac{d[DOC]}{dt} = \text{net DOC production rate} - \text{dilution rate}$$

$$\frac{d[BioC]}{dt} = \text{Inorganic carbon uptake rate} - \text{net DOC production rate} - \text{dilution rate}$$

Because we fixed the dilution rate of each flask at 1/30 per day, C removal due to daily dilution from each carbon pool was 1/30 of the pool size per day. At steady state, we assumed that all C pools remained at constant concentrations, and by setting the above differential equations equal to 0, we calculated net C uptake rate and net DOC production rate. We expressed all calculated carbon flux rates as changes in concentration per day:

$$\text{Net DOC production rate}(\mu\text{mol per Liter per day}) = \frac{[DOC]}{30}$$

$$\text{Net photosynthetic inorganic carbon uptake rate } (\mu\text{mol per Liter per day}) = \frac{[BioC] + [DOC]}{30}$$

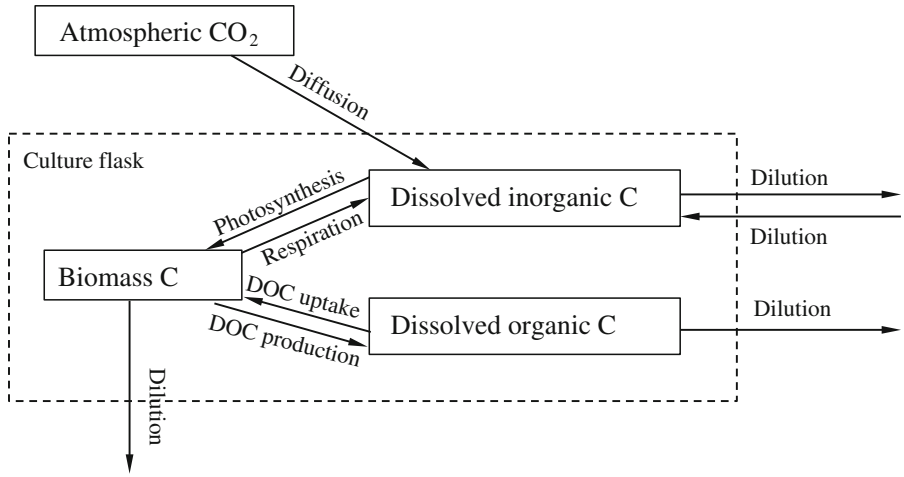

Fig. 1 Schematic diagram of carbon pools and carbon fluxes in the experimental systems

Statistical analyses

We performed multi-way analysis of variance to analyze the effects of varying CO_2, N and P on chlorophyll *a* concentrations, total cell volumes, DOC and BioC pool sizes, inorganic C uptake rates, and C/N and C/P ratios in biomass and dissolved organic matter (DOM). We log-transformed each response variable prior to statistical analysis to homogenize variance. As outlined above, net DOC production rate is proportional to DOC concentration, and we thus only report the results of ANOVA for DOC pool size. All statistical analyses were performed in R, version 2.15.0 (R Development Core Team 2011).

Results

Culture growth

All but one of the cultures reached a steady state, defined by stationary OD after 38 days of dilution. Only one flask (40 µmol L^{-1} N, 16 µmol L^{-1} P, and 380 ppm CO_2) was still accumulating biomass at the time of harvest. Our microscope counts revealed that this flask was dominated by the N_2-fixing cyanobacterium *Anabaena* sp. and we excluded it from all statistical analyses because it was not at steady state.

Carbon dynamics

The steady-state pH of cultures continuously exposed to 380 and 700 ppm CO_2 was 7.29 and 7.20 respectively. This difference in pH is less than the ~0.2–0.4 unit change predicted by Caldeira and Wickett (2003) due to the buffering capacity of growth medium. Assuming chemical equilibrium and diffusion equilibrium with the atmosphere, we calculated inorganic C concentration in the experimental cultures as a function of pH and temperature. Calculated concentrations of dissolved CO_2 were 14.82 and 27.30 µmol L^{-1} in the 380 and 700 ppm CO_2 treatments, respectively. Bicarbonate was the main form of DIC in both CO_2 treatments, comprising 89.02 % (at 380 ppm CO_2) and 86.86 % (at 700 ppm CO_2) of total DIC. Total DIC concentrations under 380 and 700 ppm CO_2 were 135.94 and 208.63 µmol L^{-1}, respectively.

Algal C dynamics exhibited a mix of responses to our experimental manipulations. Chlorophyll *a* concentrations responded positively to increasing N availability, but responded negatively to increasing CO_2 (Fig. 2d; Tables 1, 2). Total phytoplankton cell volume was significantly higher when N availability was high. BioC, as calculated from cell volume and the empirical relationship between phytoplankton biomass C and bacterial biomass C (see "Materials and methods" section), similarly showed a strong positive response to increasing N availability (Fig. 2b; Tables 1, 2). However, we did not find significant effects of elevated CO_2

on BioC (Fig. 2b; Tables 1, 2). In contrast, we observed significantly higher DOC concentrations under 700 ppm CO_2 treatment (Fig. 2c; Table 1, 2), and this enhancement was consistent across the nutrient treatments. Surprisingly, nutrient treatments did not significantly change DOC concentrations (Fig. 2c; Table 1). Finally, both elevated CO_2 levels and high N availability (160 μM) significantly stimulated the net photosynthetic C uptake rate (Fig. 2a; Tables 1, 2).

C/nutrient stoichiometry

Dissolved inorganic N and P concentrations at steady state were low in general ([nitrate] < 6.3 μmol L^{-1}, and [phosphate] < 1 μmol L^{-1}), and did not vary much across treatments. Thus, most of the supplied N and P was taken up by phytoplankton and bacteria. As a consequence, high input N and P concentrations resulted in significantly lower biomass C/N and C/P ratio respectively (Fig. 3a, b; Tables 1, 2). However, varying inorganic N and P availability did not significantly influence C/N or C/P in DOM (Tables 1, 2). The influence of elevated CO_2 on biomass stoichiometry was not pronounced but the C/N and C/P ratios of DOM were both significantly higher under elevated CO_2 (Tables 1, 2). For example, the C/N molar ratio in DOM averaged 6.34 and 21.99 at 380 ppm CO_2 and 700 ppm CO_2, respectively. Similarly, the C/P molar ratio in DOM averaged 572.23 and 1,374.81 at 380 and 700 ppm CO_2, respectively.

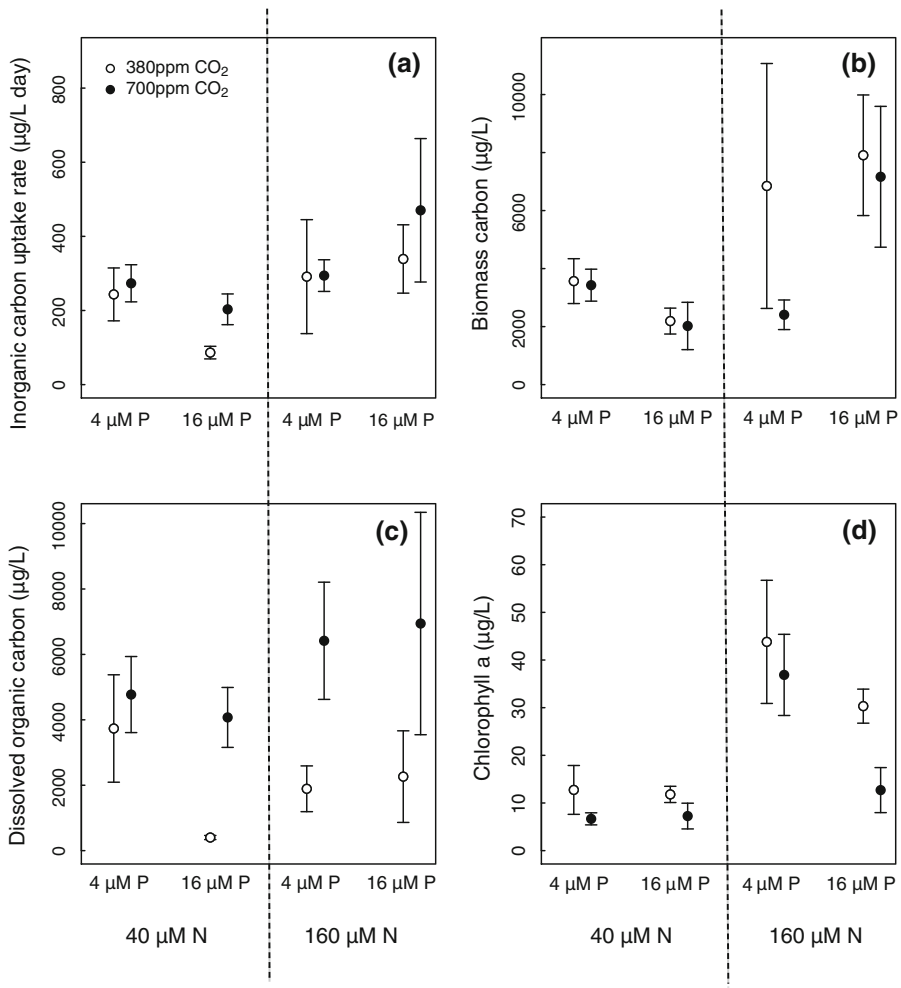

Fig. 2 Carbon pool sizes, carbon flux rates, and chlorophyll *a* concentrations at steady state. **a** Inorganic carbon uptake rate (net photosynthesis); **b** biomass carbon; **c** dissolved organic carbon; and **d** chlorophyll *a*. The error bars indicate one standard error

Table 1 *p*-value of analysis of variance showing the effects of treatments and their interaction on each response variable

	CO_2	N	P	CO_2:N	CO_2:P	N:P	CO_2:N:P
DOC	<0.001*(+)	0.17	0.091	0.75	0.062	0.037*	0.14
Dissolved organic matter C/N	0.003*(+)	0.63	0.73	0.40	0.43	0.17	0.75
Dissolved organic matter C/P	0.003*(+)	0.61	0.40	0.92	0.067	0.63	0.082
BioC	0.34	0.014*(+)	0.69	0.65	0.61	0.012*	0.33
Biomass C/N	0.22	<0.001*(−)	0.74	0.91	0.46	0.025*	0.55
Biomass C/P	0.33	0.014*(+)	<0.001*(−)	0.68	0.61	0.011*	0.32
Inorganic carbon uptake rate	0.05*(+)	0.012*(+)	0.63	0.76	0.30	0.018	0.57
Chlorophyll *a*	0.046*(−)	<0.001*(+)	0.31	0.63	0.54	0.19	0.77
Total cell volume	0.19	0.044*(+)	0.55	0.79	0.84	0.007*	0.36

(*) indicates a significant effect at 5 % significance level. When the treatment has a significant effect, (+) and (−) indicates positive and negative effects respectively

Discussion

Enhanced inorganic C uptake and accumulation of DOC under elevated CO_2

Our experiment showed that continuously elevated atmospheric CO_2 significantly enhanced the DOC production rate in experimental microcosms populated by phytoplankton and bacteria (Fig. 2c; Table 1). We also found statistically significant increases in inorganic C uptake rate under the high CO_2 treatment, although the pattern was not as pronounced (Fig. 2a; Table 1). The fact that chlorophyll *a* concentrations decreased under elevated CO_2 means that the apparently enhanced rate of C fixation under elevated CO_2 was the result of an increased rate of photosynthetic C uptake per unit mass of chlorophyll *a*. Such an increase in photosynthetic efficiency under elevated CO_2 has been observed in previous studies using single species algal cultures, such as the cyanobacterium *Spirulina platensis* (Gordillo et al. 1999), the diatom *Thalassiosira pseudonana* (Sobrino et al. 2008), and the coccolithophorid *Emiliania huxleyi* (Lefebvre et al. 2012), as well as in natural mixed-species plankton communities (Riebesell et al. 2007).

Although inorganic C has historically been considered abundant for primary production in aquatic ecosystems (Raven and Johnston 1991; Falkowski 1994), evidence for CO_2-stimulated productivity in aquatic ecosystems is increasing. This evidence is consistent with our current understanding of phytoplankton inorganic carbon uptake mechanisms. The intracellular carboxylation enzymes in most phytoplankton species require 25–35 μmol L^{-1} CO_2 to saturate (Raven and Johnston 1991), but at current atmospheric concentrations of 380 ppm CO_2 and ambient temperatures, the equilibrium concentration of dissolved CO_2 in water is only about 10 μmol L^{-1}, a value that is insufficient to saturate carboxylation. This can result in potential C limitation for photosynthetic C fixation, especially for phytoplankton species that cannot concentrate CO_2 internally by actively transporting and converting HCO_3^- (Riebesell et al. 1993). For example, photosynthetic carboxylation in phytoplankton species without C concentrating mechanisms such as coccolithophorids *Emiliania huxleyi* (Engel et al. 2005) and *Gephyrocapsa oceanica* (Riebesell et al. 2000), can be far below saturation under current atmospheric CO_2 concentrations. Even for algal species possessing C concentrating mechanisms which enable abundant HCO_3^- to be converted to CO_2 via β-carbonic anhydrase, elevated CO_2 could still stimulate C fixation because the C concentrating process is often limited by available energy (Riebesell 2004) or by the availability of enzyme cofactors such as zinc (Morel et al. 1994). Thus, an increase in dissolved CO_2 due to elevated atmospheric CO_2 essentially allows more energy efficient C acquisition (Raven 1991).

Under our experimental conditions, carbonate chemistry predicts that dissolved CO_2 in equilibrium with the atmosphere should increase from 14.82 μmol L^{-1} at 380 ppm atmospheric CO_2 to a level of 27.30 μmol L^{-1} at 700 ppm atmospheric CO_2. The significant increase in dissolved CO_2 may have increased the concentration

Table 2 Mean and standard error of measurement in each CO_2, N and P treatment combination

	380 ppm CO_2				700 ppm CO_2			
	40 μM N		160 μM N		40 μM N		160 μM N	
	4 μM P	16 μM P	4 μM P	16 μM P	4 μM P	16 μM P	4 μM P	16 μM P
Inorganic C uptake rate (μg/L day)	243.42 ± 71.36	86.39 ± 17.00	291.31 ± 153.73	339.00 ± 92.20	273.37 ± 50.06	203.18 ± 41.48	294.14 ± 42.73	470.31 ± 193.46
BioC (μg/L)	3,567.83 ± 772.97	2,190.76 ± 446.44	6,848.10 ± 4,221.45	7,906.82 ± 2,078.76	3,428.68 ± 550.98	2,021.93 ± 815.97	2,407.93 ± 510.20	7,165.12 ± 2,427.39
DOC (μg/L)	3,734.74 ± 1,642.00	410.02 ± 63.55	1,891.19 ± 699.72	2,263.25 ± 1,400.51	4,772.39 ± 1,163.01	4,073.33 ± 916.33	6,416.40 ± 1,791.95	6,944.23 ± 3,399.86
Chl *a* (μg/L)	12.74 ± 5.12	11.80 ± 1.70	43.81 ± 12.92	30.32 ± 3.57	6.67 ± 1.27	7.26 ± 2.70	36.88 ± 8.52	12.70 ± 4.73
Biomass C/N	34.79 ± 6.01	19.87 ± 5.13	4.49 ± 2.77	4.98 ± 1.30	23.78 ± 0.95	19.56 ± 8.73	1.58 ± 0.33	17.76 ± 12.59
Biomass C/P	94.85 ± 21.33	12.23 ± 2.56	174.96 ± 105.76	43.55 ± 11.51	89.73 ± 13.18	11.16 ± 4.53	62.27 ± 12.55	67.77 ± 23.10
DOM C/N	10.98 ± 5.05	7.80 ± 6.57	5.23 ± 1.91	7.92 ± 5.05	16.93 ± 4.38	12.67 ± 2.35	18.52 ± 5.49	30.44 ± 17.52
DOM C/P	1,284.37 ± 701.35	302.42 ± 263.17	363.44 ± 154.75	338.71 ± 211.74	811.39 ± 206.29	2,291.35 ± 656.50	1,174.80 ± 308.33	1,974.77 ± 423.27

Data are shown as mean ± standard error, element ratios are expressed as molar ratios

of dissolved CO_2 enough to stimulate primary production. This provides a possible explanation for enhanced photosynthetic C fixation under elevated CO_2 even though inorganic C is abundant in aquatic ecosystems at current CO_2 levels.

Our experimental results are also consistent with the evidence for enhanced net primary production under elevated atmospheric CO_2 that have been reported in other studies. A relatively recent short-term CO_2 perturbation experiment with marine phytoplankton communities grown in large mesocosoms resulted in a 39 % increase in total C consumption at 1,050 ppm CO_2 relative to ambient CO_2 levels (Riebesell et al. 2007). Yue and Chen (2005) observed more than a doubling of *Chlorella* biomass when CO_2 concentrations were increased to 10 % (volume ratio). Similarly, during incubations of natural phytoplankton assemblages obtained from a nutrient-poor central Atlantic transect, C fixation was enhanced by up to 15 % in response to a threefold increase in CO_2 concentration (Hein and Sand-Jensen 1997). Even more remarkably, Jansson et al. (2012) recently demonstrated that rates of phytoplankton primary production were up to ten-fold higher in CO_2-supersaturated lake water, relative to water containing CO_2 at normal equilibrium concentrations. Our results differ importantly from previous studies in that we observed a significant CO_2 effect on phytoplankton net primary production under continuous CO_2 enrichment. Combined with results from previous studies, our data suggest that a significant enhancement in net photosynthetic carbon uptake can occur in response to continuously elevated atmospheric CO_2.

A sustained increase in photosynthetic C uptake rate in aquatic ecosystems has the potential to sequester additional C and buffer increasing atmosphere CO_2 to some extent, even if N and P availability does not increase. We observed significant positive effects of both N availability and CO_2 on photosynthetic C fixation rate (Table 1), but we did not find a statistically significant CO_2 × nutrients interaction on any C pool size and flux rate (Table 1). We did not observe evidence that CO_2 fertilization effects depend on nutrient availability. Although this could result from low statistical power due to low replication, it is consistent with previous observations that CO_2 enrichment can result in excess C fixation independent of nutrient availability

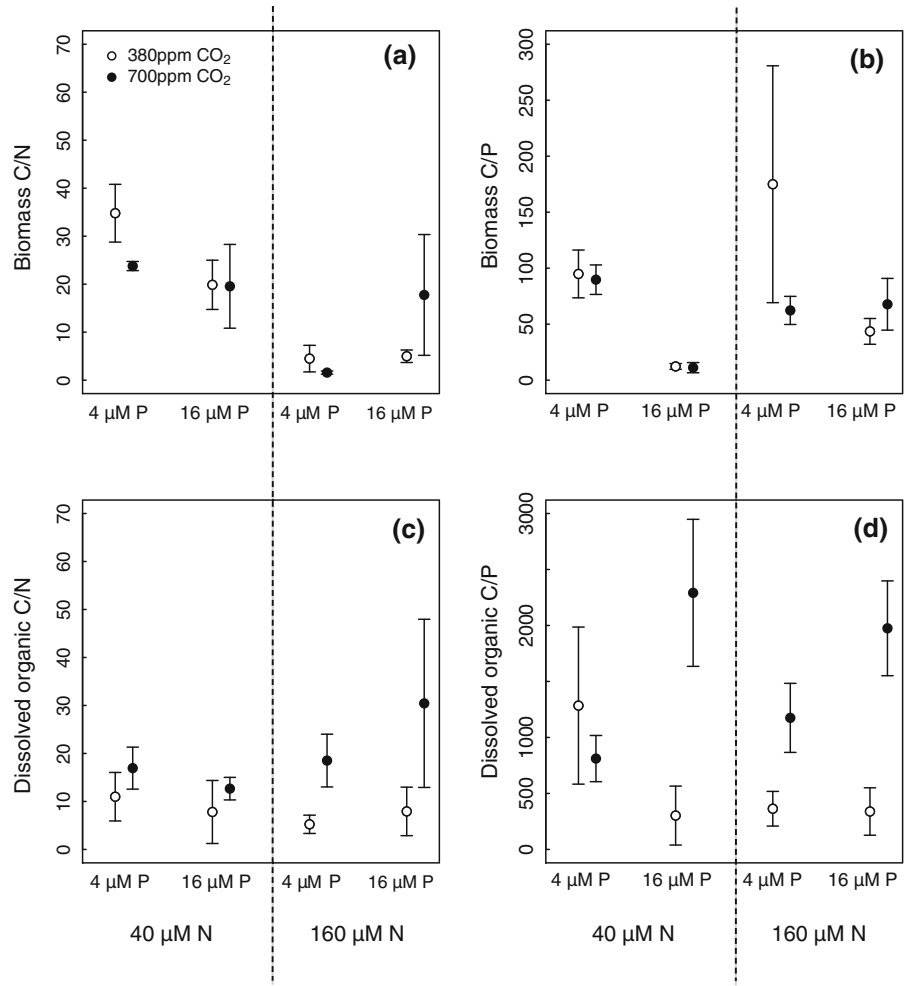

Fig. 3 Steady-state C/N and C/P stoichiometry. **a** C/N ratios in biomass; **b** C/P ratios in biomass; **c** C/N ratios in dissolved organic matter and **d** C/P ratios in dissolved organic matter. All ratios are expressed in moles. The error bars indicate one standard error

in aquatic ecosystems (Hessen et al. 2004; Hessen and Anderson 2008). Previous studies have observed various fates of the excess C. For example, Leonardos and Geider (2005) found that increased atmospheric CO_2 concentration enhanced particulate organic C production under nutrient limited conditions. Borchard and Engel (2012) observed a significant increase in organic matter exudation of *Emiliania huxleyi* under elevated CO_2 and controlled P conditions. In our study, we only observed enhanced DOC production, and not an increase in biomass under elevated CO_2. Together, these results suggest that excess C uptake relative to nutrients could potentially be sustained under continuous CO_2 enrichment, but the ultimate fate of excess C could be highly variable.

Enhanced DOC production with shifting C/nutrient stoichiometry

The dramatically enhanced steady state rates of DOC production under elevated CO_2 (Fig. 2c) may have implications for long-term C storage in aquatic ecosystems. Understanding the dynamics of DOC is important because DOC plays a significant role in the creation of sinking biogenic particles and in the formation of recalcitrant DOC. First, DOC is an important precursor for the formation of transparent exopolymetric particles (TEPs) (Passow 2000). Owing to their surface-reactive nature, TEP support coagulation processes and enhance the formation of large particle aggregates (Passow 2002). The formation of TEP is an important pathway to convert dissolved into

particulate organic C (Engel et al. 2004), and subsequently facilitates vertical transport of particulate organic C below the mixed layer (Alldredge et al. 1993). Enhanced TEP production in a high CO_2 environment has been observed in natural phytoplankton communities (Engel 2002) as well as in monospecific cultures of the diatom *Thalassiosira weissflogii* and the coccolithophorid *Emiliania huxleyi* (Engel et al. 2005). The increased rates of DOC production we observed under elevated CO_2 provide a potential explanation for increasing TEP results from increased C availability. Second, DOC itself is a large C pool and an important potential C reservoir (Hansell et al. 2009). Recalcitrant DOC can be formed from labile C via heterotrophic metabolism, and can form a long term C reservoir in the upper layers of aquatic ecosystems (Jiao et al. 2010). In a 36 day incubation of *Pseudomonas chlororaphis*, the only C source, D-glucose, was consumed within 2 days but 5–10 % of the C derived from D-glucose persisted until the end of the experiment (Gruber et al. 2006). A year long incubation of marine bacterial assemblages showed that up to 50 % of C derived from labile C sources persisted until the end of the incubation, indicating that heterotrophic bacteria can generate relatively stable C from labile sources (Ogawa et al. 2001). Since DOC can be transformed to recalcitrant forms by various pathways, the enhanced DOC production at steady state observed in our experiment also suggests the potential for enhanced flux into the large and relatively stable refractory DOC reservoir in aquatic ecosystems.

Finally, the elemental stoichiometry we computed allowed us to infer the recalcitrance to of DOC. Hopkinson and Vallino (2005) showed that C/N/P stoichiometry of relatively recalcitrant deep-water DOM (molar ratio of C/N/P = 3511/202/1) is dramatically different from that in relatively labile, newly produced DOM (199/20/1). In our experiment, the C/N ratio and C/P ratio of DOM increased significantly in the high CO_2 treatments (Fig. 3c; Tables 1, 2). The mean C/N and C/P ratio of DOM (Table 2) under high CO_2 was comparable to the stoichiometry of recalcitrant DOM (3511/202/1) reported by Hopkinson and Vallino (2005), suggesting a possible shift in the production of relatively labile DOC under ambient CO_2 to the production of more recalcitrant DOC under elevated CO_2. Such a shift is consistent with an increase in the stability of DOC with rising CO_2. However, C/N and C/P stoichiometry is only a rough indicator of stability. Detailed molecular characterization is needed to more decisively evaluate the stability of DOC. Regardless, our experiment demonstrates that continuous CO_2 enrichment enhances DOC production and that newly produced DOC has higher C/nutrient ratio. Further analyses are needed to confirm the biological stability of this newly generated DOC.

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References

Alldredge AL, Passow U, Logan BE (1993) The abundance and significance of a class of large, transparent organic particles in the ocean. Deep Sea Res 40(6):1131–1140

Ballantyne F, Menge D, Ostling A, Hosseini P (2008) Nutrient recycling affects autotroph and ecosystem stoichiometry. Am Nat 171(4):511–523

Bauer JE, Williams PM, Druffel ERM (1992) ^{14}C activity of dissolved organic carbon fractions in the north-central Pacific and Sargasso Sea. Nature 357(6380):667–670

Borchard C, Engel A (2012) Organic matter exudation by *Emiliania huxleyi* under simulated future ocean conditions. Biogeoscience 9(8):3405–3423

Caldeira K, Wickett ME (2003) Anthropogenic carbon and ocean pH. Nature 425(6956):365

Campbell W, Song P, Barbier G (2006) Nitrate reductase for nitrate analysis in water. Environ Chem Lett 4(2):69–73

Cole JJ, Prairie YT, Caraco NF, McDowell WH, Tranvik LJ, Striegl RG, Duarte CM, Kortelainen P, Downing JA, Middelburg JJ, Melack J (2007) Plumbing the global carbon cycle: integrating inland waters into the terrestrial carbon budget. Ecosystems 10(1):172–185

Ducklow HW, Steinberg DK, Buesseler KO (2001) Upper ocean carbon export and the biological pump. Oceanography 14(4):50–58

Elrifi IR, Turpin DH (1986) Nitrate and ammonium induced photosynthetic suppression in N-limited *Selenastrum minutum*. Plant Physiol 81(1):273–279

Elser JJ, Bracken MES, Cleland EE, Gruner DS, Harpole WS, Hillebrand H, Ngai JT, Seabloom EW, Shurin JB, Smith JE (2007) Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecol Lett 10(12):1135–1142

Engel A (2002) Direct relationship between CO_2 uptake and transparent exopolymer particles production in natural phytoplankton. J Plankton Res 24(1):49–53

Engel A, Thoms S, Riebesell U, Rochelle-Newall E, Zondervan I (2004) Polysaccharide aggregation as a potential sink of marine dissolved organic carbon. Nature 428(6986):929–932

Engel A, Zondervan I, Aerts K et al (2005) Testing the direct effect of CO_2 concentration on a bloom of the coccolithophorid *Emiliania huxleyi* in mesocosm experiments. Limnol Oceanogr 50(2):493–507

Eppley RW, Peterson BJ (1979) Particulate organic matter flux and planktonic new production in the deep ocean. Nature 282(5740):677–680

Falkowski PG (1994) The role of phytoplankton photosynthesis in global biogeochemical cycles. Photosynth Res 39(3): 235–258

Falkowski PG, Raven JA (2007) Aquatic photosynthesis. Princeton University Press, Princeton

Geider RJ, Delucia EH, Falkowski PG et al (2001) Primary productivity of planet earth: biological determinants and physical constraints in terrestrial and aquatic habitats. Glob Change Biol 7(8):849–882

Gordillo FJL, Jiménez C, Figueroa FL, Niell FX (1999) Effects of increased atmospheric CO_2 and N supply on photosynthesis, growth and cell composition of the cyanobacterium *Spirulina platensis* (*Arthrospira*). J Appl Phycol 10(5): 461–469

Gruber DF, Simjouw JP, Seitzinger SP, Taghon GL (2006) Dynamics and characterization of refractory dissolved organic matter produced by a pure bacterial culture in an experimental predator–prey system. Appl Environ Microbiol 72(6):4184–4191

Guillard RRL (1975) Culture of phytoplankton for feeding marine invertebrates. In: Smith WL, Chantey MH (eds) Culture of marine invertebrate animals. Plenum Publishers, New York, pp 29–60

Halterman SG, Toetz DW (1984) Kinetics of nitrate uptake by freshwater algae. Hydrobiologia 114(3):209–214

Hansell DA, Carlson CA, Repeta DJ, Schlitzer R (2009) Dissolved organic matter in the ocean: a controversy stimulates new insights. Oceanography 22(4):202–211

Harpole WS, Ngai JT, Cleland EE et al (2011) Nutrient colimitation of primary producer communities. Ecol Lett 14(9):852–862

Hein M, Sand-Jensen K (1997) CO_2 increases oceanic primary production. Nature 388(6642):526–527

Hessen DO (2008) Efficiency, energy and stoichiometry in pelagic food webs: reciprocal roles of food quality and food quantity. Freshw Rev 1(1):43–57

Hessen DO, Anderson TR (2008) Excess carbon in aquatic organisms and ecosystems: physiological, ecological and evolutionary implications. Limnol Oceanogr 53(4): 1685–1696

Hessen DO, Ågren GI, Anderson TR (2004) Carbon sequestration in ecosystems: the role of stoichiometry. Ecology 85(5):1179–1192

Hopkinson CS, Vallino JJ (2005) Efficient export of carbon to the deep ocean through dissolved organic matter. Nature 433(7022):142–145

Hopkinson BM, Xu Y, Shi D, McGinn PJ, Morel FMM (2010) The effect of CO_2 on the photosynthetic physiology of phytoplankton in the Gulf of Alaska. Limnol Oceanogr 55(5):2011–2024

Hutchins D, Fu FX, Zhang Y, Warner ME, Feng Y, Portune K, Bernhardt PW, Mulholland MR (2007) CO_2 control of *Trichodesmium* N_2 fixation, photosynthesis, growth rates, and elemental ratios: implications for past, present, and future ocean biogeochemistry. Limnol Oceanogr 52(4): 1293–1304

Jansson M, Karlson J, Jonsson A (2012) Carbon dioxide supersaturation promotes primary production in lakes. Ecol Lett 15(6):527–532

Jiao N, Herndl GJ, Hansell DA et al (2010) Microbial production of recalcitrant dissolved organic matter: long-term carbon storage in the global ocean. Nat Rev Microbiol 8(8):593–599

Kilham P, Hecky RE (1988) Comparative ecology of marine and freshwater phytoplankton. Limnol Oceanogr 33(4):776–795

Langner CL, Hendrix PF (1982) Evaluation of a persulfate digestion method for particulate nitrogen and phosphorus. Water Res 16(10):1451–1454

Lefebvre SC, Benner I, Stillman JH, Parker AE, Drake MK, Rossignol PE, Okimura KM, Komada T, Carpenter EJ (2012) Nitrogen source and pCO_2 synergistically affect carbon allocation, growth and morphology of the coccolithophore *Emiliania huxleyi*: potential implications of ocean acidification for the carbon cycle. Glob Change Biol 18(2):493–503

Leonardos N, Geider RJ (2005) Elevated atmospheric carbon dioxide increases organic carbon fixation by *Emiliania huxleyi* (Haptophyta), under nutrient-limited high-light conditions. J Phycol 41(6):1196–1203

Menden-Deuer S, Lessard EJ (2000) Carbon to volume relationships for dinoflagellates, diatoms, and other protist plankton. Limnol Oceanogr 45(3):569–579

Morel FMM, Reinfelder JR, Roberts SB, Chamberlain CP, Lee JG, Yee D (1994) Zinc and carbon co-limitation of marine phytoplankton. Nature 369(6483):740–742

Ogawa H, Amagai Y, Koike I, Kaiser K, Benner R (2001) Production of refractory dissolved organic matter by bacteria. Science 292(5518):917–920

Passow U (2000) Formation of transparent exopolymer particles, TEP, from dissolved precursor material. Mar Ecol Prog Ser 192:1–11

Passow U (2002) Transparent exopolymer particles (TEP) in aquatic environment. Prog Oceanogr 55(3–4):287–333

Peeters F, Straile D, Lorke A, Livingstone DM (2007) Earlier onset of the spring phytoplankton bloom in lakes of the temperate zone in a warmer climate. Glob Change Biol 13(9):1898–1909

R Development Core Team (2011) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Raven JA (1991) Physiology of inorganic C acquisition and implications for resource use efficiency by marine phytoplankton: relation to increased CO_2 and temperature. Plant Cell Environ 14(8):779–794

Raven JA, Johnston AM (1991) Mechanisms of inorganic-carbon acquisition in marine phytoplankton and their implications for the use of other resources. Limnol Oceanogr 36(8):1701–1714

Riebesell U (2004) Effects of CO_2 enrichment on marine phytoplankton. J Oceanogr 60(4):719–729

Riebesell U, Wolf-Gladrow DA, Smetacek V (1993) Carbon dioxide limitation of marine phytoplankton growth rates. Nature 361(6409):249–251

Riebesell U, Zondervan I, Rost B, Tortell PD, Zeebe RE, Morel FMM (2000) Reduced calcification of marine plankton in

response to increased atmospheric CO_2. Nature 407(6802): 364–367

Riebesell U, Schulz K, Bellerby R et al (2007) Enhanced biological carbon consumption in a high CO_2 ocean. Nature 450(7169):545–548

Sabine CL, Feely RA, Gruber N et al (2004) The oceanic sink for anthropogenic CO_2. Science 305(5682):367–371

Sarmiento J, Slater R, Barber R et al (2004) Response of ocean ecosystems to climate warming. Glob Biogeochem Cycles 18(3):GB3003

Sartory DP, Grobbelaar JU (1984) Extraction of chlorophyll a from freshwater phytoplankton for spectrophotometric analysis. Hydrobiologia 114(3):177–187

Schindler D (1977) Evolution of phosphorus limitation in lakes. Science 195(4275):260–262

Schlesinger WH (1997) Biogeochemistry: an analysis of global change. Academic Press, San Diego

Simon M, Cho BC, Azam F (1992) Significance of bacterial biomass in lakes and the ocean: comparison to phytoplankton biomass and biogeochemical implications. Mar Ecol Prog Ser 86:103–110

Smith VH (1979) Nutrient dependence of primary productivity in lakes. Limnol Oceanogr 24(6):1051–1064

Smith VH (2006) Responses of estuarine and coastal marine phytoplankton to nitrogen and phosphorus enrichment. Limnol Oceanogr 51(1):377–384

Sobrino C, Ward ML, Neale PJ (2008) Acclimation to elevated carbon dioxide and ultraviolet radiation in the diatom *Thalassiosira pseudonana*: effects on growth, photosynthesis, and spectral sensitivity of photoinhibition. Limnol Oceanogr 53(2):494–505

Sun J, Liu D (2003) Geometric models for calculating cell biovolume and surface area for phytoplankton. J Plankton Res 25(11):1331–1346

van de Waal D, Verschoor A, Verspagen J, Van Donk E, Huisman J (2010) Climate-driven changes in the ecological stoichiometry of aquatic ecosystems. Front Ecol Environ 8(3):145–152

Van Veldhoven PP, Mannaerts GP (1987) Inorganic and organic phosphate measurements in the nanomolar range. Anal Biochem 161(1):45–48

Yue L, Chen W (2005) Isolation and determination of cultural characteristics of a new highly CO_2 tolerant fresh water microalgae. Energy Convers Manage 46(11–12):1868–1876