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Responses of the diatom *Asterionellopsis glacialis* to increasing sea water CO_2 concentrations and the effect of turbulence

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Abstract

Emissions of greenhouse gases, such as carbon dioxide (CO_2), are lead to increasing global and surface ocean temperatures. At the same time, as CO_2 equilibrates between the atmosphere and the surface ocean, it decreases sea water pH. As a result, the changes in physical and chemical properties of the ocean can affect marine primary producers in various ways. A number of researches have addressed the effects of ocean acidification on marine phytoplankton. However, phytoplankton responses to combined effects are still poorly understood. Here, we chose monospecific cultures of the cosmopolitan chain forming diatom *Asterionellopsis glacialis* (*A. glacialis*), grown semi-continuously under controlled laboratory conditions, to assess the combined effect of ocean acidification (~420 to 2800 µatm) and turbulence. At current CO_2 levels, growth rates of *A. glacialis* increased under enhanced turbulence. This was the result of an optimum shift towards lower CO_2 concentrations and accompanied by a prevalence of longer chains (more than 6 cells). For increasing CO_2 levels (up to ~2800 µatm) and decreased pH values, enhanced turbulence significantly decreased growth rates, chain length and organic matter production of *A. glacialis*. Thus, our study suggests that, even though *A. glacialis* benefited from enhanced turbulence, at present carbon dioxide concentration, at higher CO_2 levels, turbulence magnified the stress by acidification. If in the future, the ocean surface layer will be more frequently exposed to storm and wind events, then phytoplankton communities might be more sensitive to lower pH, with potential consequences for community composition and productivity.

Keywords: *Asterionellopsis glacialis*, diatoms, ocean acidification, CO_2, turbulence.

1 Introduction

Since the beginning of the Industrial Revolution, human activities such as the burning of fossil fuels, changes in land use and deforestation, led to a considerable increase of carbon dioxide concentrations in the atmosphere (Le Quéré et al., 2015). Atmospheric carbon dioxide levels have increased steadily from pre-industrial levels of approximately 280 µatm to 400 µatm today, which is above the upper limit of the last 2 million years (Hönisch et al., 2009). This increase translates into a number of global scale changes, such as the modification of the carbon cycle and the rise of global average temperatures, with potential consequences on the ecosystems level (IPCC, 2014). The ocean is the largest sink of carbon and heat mitigating both the effects of anthropogenic CO_2 rise in the atmosphere (Sarmiento and Gruber, 2002) and global warming. During the past century, more than one third of the carbon dioxide emitted into the atmosphere has been absorbed by the ocean (Sabine et al., 2004) causing unprecedented changes in sea water carbonate chemistry (Pespeni et al., 2013). As a consequence, dissolved inorganic carbon (DIC) and bicarbonate ions (HCO_3^-) have increased in the surface ocean while carbonate ions (CO_3^{2-}) and pH (Wolf-Gladrow et al., 1999) have decreased. For example, since the Industrial revolution, the pH of the surface ocean has already decreased by 0.1 units (Rhein et al., 2013) and is projected to drop by an additional 0.4 units in a "business as usual" CO_2 emission scenario by the year 2100 (Raupach et al., 2007; IPCC, 2014), shifting the carbonate equilibrium of the sea water towards more acidic conditions, while CO_2 is expected to reach up to 1000 µatm (IPCC, 2014). At the same time, the increase in global average temperature is having a number of indirect effects on the ocean. Heating of the ocean's surface acts to enhance the stratification of the surface waters, shoaling the upper mixed layer (Doney, 2006; Rost et al., 2008) and stabilizing the water column. Concomitantly, this warming is also expected to increase water vapor in the atmosphere, increasing the frequency and intensity of storm and wind events which, in turn, enhance shear turbulence in the very surface layer. (Moum and Smyth, 2001). Thus, the future ocean would be characterized by an overall water column stabilization accompanied by an increase of destabilization events (D'Asaro et al., 2014). These changes in physical properties of the ocean, caused by anthropogenic disturbances, may interact with ocean acidification and have the potential to affect community composition of marine phytoplankton assemblages with potential feedbacks to marine biogeochemical element cycling.

In the last decades numerous experiments have been adressing potential responses of marine phytoplankton to elevated CO_2 levels (e.g. Gao et al., 2012a). Particularly, the potential influence of enhanced CO_2 concentrations on calcyfing phytoplankton (coccolithophores) which are thought to be higly sensitive to ocean acidification, have been investigated intensively (Meyer and Riebesell, 2015). In comparison, only a few studies assessed the physiological response of the silica shielded phytoplankton, diatoms, (i.e: Sarthou et al., 2005; Roberts et al., 2007; Tortell et al., 2008; Wu et al., 2015; Clement et al., 2016) even though they contribute with up to 40 % of marine primary production in the ocean and are responsible for a large portion or organic carbon export to the deep ocean (Ducklow et al., 2001; Scott, 2005; Hopkinson et al., 2011). Furthermore, responses of diatoms to varying CO_2 levels range from positive in terms of increased growth and carbon fixation rates, and photosynthetic efficiency (Chen and Gao 2003; Chen and Gao, 2004; Wu et al., 2010; McCarthy et al., 2012, Barcelos e Ramos et al., 2014) to absent or negative responses (Burkhardt et al., 1999; Crawfurd et al., 2011) even under comparable experimental conditions.

In addition, , phytoplankton cells occurring in a shallower mixed layer will be more frequently at the top layer of the surface ocean and therefore more exposed to destabilization events (D'Asaro et al., 2014). However, the effects of these changes of the oceans physical characteristics on phytoplankton have been poorly investigated (Garrison and Tang, 2014). Especially, most of the knowledge concerning the effects of turbulent environments on phytoplankton focused on phytoplankton communities (Estrada et al., 1987; Peters and Marrasè, 2000; Barton et al., 2014; Zhou et al., 2015), with only a few studies investigating the response of individual species (Berdalet and Estrada, 1993; Berdalet et al., 2007; Garrison and Tang, 2014). It has been reported that enhanced turbulence can benefit large cells by increasing the nutrient flux to their surface since the water motion has the potential to overcome the limits of diffusive transport of nutrients whereas smaller cells should not be affected (Gavis, 1976; Lazier and Mann 1989; Karp-Boss et al., 1996; Peters et al. 2006; Guasto et al. 2012). However, disrupting the diffusive boundary layer that surrounds the cells exposes them to the chemical conditions of bulk seawater. In our study, we chose the cosmopolitan chain forming diatom *Asterionellopsis glacialis*, to assess the combined effects of enhanced turbulence and CO_2 on physiological rates in terms of cell growth, organic matter production, cellular elemental quotas and chain formation.

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2 Material and methods

2.1 Experimental Set-up

Monospecific cultures of the cosmopolitan *A. glacialis* (strain CCMMG_1 isolated in 2011 from offshore Terceira Island, Azores) were grown in sterile filtered (0.2 µm) North Atlantic sea water (salinity 35.9 psu sea water) enriched with approximately 4.5 µmol L^{-1} of phosphate, 64 µmol L^{-1} of nitrate and silicate, and trace metals and vitamins according to the f/20 medium (Guillard and Ryther, 1962). Cultures were acclimated for at least 18 generations at low abundances (average final concentration of less than 15000 cells ml^{-1}), to avoid significant changes in sea water carbonate chemistry speciation. All cultures were grown semi-continuously at 20 ºC, constant light intensity (incident photon flux density of ~170 ± 10 µmol m^{-2} s^{-1}), 14/10 hours light/dark cycle under control and enhanced turbulence conditions and at four CO_2 levels (ranging approximately from 420 to 2800 µatm, corresponding to pH_T values (total scale) between ~8.04 and 7.3), resulting in a total of eight treatments. Culture bottles grown under control conditions were gently rotated vertically (20 times) daily in order to avoid sedimentation, whereas cultures grown under enhanced turbulence were additionally exposed to constant mixing generated by an orbital shaker with 220 rpm speed. The experiment was conducted in triplicates (for more details see Table 1).

2.2 Cell numbers and growth rates

The abundance of *A. glacialis* and the number of cells in each chain were determined from lugol fixed samples (2 % final concentration) by means of an inverted microscope (Nikon Eclipse TS100, 200 X magnifications). Cellular growth rates were determined following Levasseur et al. (1993) as:

$$\mu = \ln (Cf / Ci) / \Delta t$$

where Cf and Ci represent the final and the initial cell concentrations, respectively, and Δt corresponds to the growth period in days.

2.3 Carbonate chemistry manipulation, measurements and calculations

The carbonate system was manipulated by combined additions of HCl and $NaHCO_3$, maintaining total alkalinity constant (Schulz et al., 2009). Media carbonate chemistry speciation was calculated with the software CO2SYS (Lewis and Wallace, 1998), using measured total alkalinity, pH_T, temperature, salinity, phosphate and silicate, and the equilibrium constants determined by Mehrbach et al. (1973) as refitted by Dickson and Millero (1987). The pH_T was measured with an electrode cell (WTW 340i pH meter) and calibrated with a Tris sea water buffer (provided by A. Dickson) according to Dickson et al. (2007). Total alkalinity was measured by potentiometric titration according to Dickson et al. (2003) using a Metrohm 848 Titrino Plus equipped with Metrohm 869 Compact Sample changer. Total alkalinity measurements were corrected with certified reference material (Dickson 2010) at about 20 μmol kg^{-1} accuracy and 2 μmol kg^{-1} precision.

2.4 Cellular element quotas and production rates

At the end of the experiment samples for cellular particulate organic carbon (POC), nitrogen (PON) and phosphorus (POP) were gently filtered (200 mbar) onto pre-combusted GF/F filters (6 hours, 450 °C) and stored at −20 °C until analyses. POC and PON filters were then dried at 60 °C for 4 hours, packed in tin boats and analyzed following Sharp (1974) using an elemental analyzer (Thermo Flash EA) coupled to an isotope ratio mass spectrometer (Thermo Delta V Plus) via a Thermo Conflo V manifold. POP samples were oxidized to dissolved inorganic phosphorus with potassium peroxydisulphate and measured colorimetrically by means of a spectrophotometer (Cary 50) following Hansen and Koroleff (1999).

POC, PON and POP production rates were calculated by multiplying cellular quotas with corresponding growth rates (μ).

2.5 Dissolved Inorganic Phosphate and Silicate

Samples for the determination of dissolved inorganic phosphate and silicate concentrations, used for the calculation of the carbonate chemistry speciation, were taken at the beginning and at the end of the experiment. Samples were filtered through 0.2 µm polyethersulfone (PES) syringe filter and stored at −20 °C until being analyzed. Concentrations of dissolved inorganic silicate and phosphate were determined spectrophotometrically (Cary 50 Probe, Varian) following Hansen and Koroleff (1999).

2.6 Statistical Analysis

Statistical significance was assessed by means of one-way analysis of variance (ANOVA) with the program SigmaPlot 11.5 and the values of $p < 0.01$ by Tukey test were considered to be significant.

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3 Results

The diatom *A. glacialis* was grown at increasing CO_2 concentrations under relatively stable and enhanced turbulence conditions.

The carbonate chemistry data is presented in Table 1 and expressed in the graphs as the average between the initial and the final values of calculated pCO_2.

3.1 Growth Rate (μ)

With growth rates peaking at the second lowest CO_2 level of ~780 µatm *A. glacialis* displayed an optimum curve response in the control treatments. Particularly, we observed a significant ($p<0.001$) 26 % increase from ~420 to ~780 µatm, followed by 40 % and 22 % decrease between ~780 and ~1110 µatm and ~1110 and ~2800 µatm respectively. When exposed to enhanced turbulence, *A. glacialis* appeared to shift the optimum growth rate towards lower CO_2 concentrations (below 420 µatm). Increasing CO_2 levels from ~420 to ~780 µatm then reduced growth rates by 25 % followed by a further decrease of 48 % and 21 % from ~780 to 1110 µatm and from ~1110 to ~2800 µatm, respectively. When comparing the control with enhanced turbulence conditions, we observed that at present carbon dioxide concentrations, growth rate was 29 % lower ($p<0.001$) under control conditions. At enhanced CO_2 levels, however, cell division rates of *A. glacialis* were higher under control (19 %, 41 % and 44 % at CO_2 concentrations of 780, 1100 and 2800 µatm respectively) than under enhanced turbulence conditions (Figure 1).

3.2 Cell quotas and organic matter production

Under control conditions, cellular element quotas for POC, PON and POP were not significantly ($p>0.001$) affected by increasing CO_2 levels from ~420 to ~780 µatm. However, when CO_2 arose from approximately 780 to ~1110 µatm, cellular element quotas were significantly increased. This increase of cellular element quotas was not sustained and decreased again to the initial values in the interval between ~1110 and ~2850 µatm. However, cells in the enhanced turbulence treatment decreased their cellular elemental quotas linearly with increasing CO_2 levels. Comparing control with enhanced turbulence conditions, we observed that at CO_2 levels higher than ~780 µatm, cellular element quotas were significantly reduced by turbulence (reduction of 32 %, 35 % and 48 % of POC, PON and POP quotas respectively at CO_2 levels ~1110 µatm and reduction of ~ 58 % POC, PON and POP quotas at CO_2 levels ~2800 µatm) (Figure 2).

Carbon, nitrogen and phosphorus production rates followed the trends of growth rates. Under control conditions, carbon and nitrogen production rates peaked at a CO_2 concentration of ~780 µatm and phosphorus production rates at ~1110 µatm. When *A. glacialis* was exposed to enhanced turbulence, the organic matter production values were highest at present day CO_2 levels (~420 µatm). Thus, within similar CO_2 concentrations, enhanced turbulence was beneficial only at present carbon dioxide levels but higher CO_2 concentration negatively affected the organic matter production rates (Figure 3).

Particulate organic matter ratios (C/N/P) were not significantly affected by varying CO_2 levels nor by turbulence (data not shown).

3.2 Relative number of cells per chain

The relative number of cells per chain was strongly influenced by both increasing CO_2 levels and enhanced turbulence (Figure 4). Under control conditions, the relative abundance of colonies with more than 6 cells increased from 7 % at ~420 µatm to 60 % at ~2850 µatm at expense of chains composed of 1 to 3 cells which decreased linearly from 67 % at ~420 µatm to 15 % at ~2850 µatm; meanwhile no change in abundance of chains comprising 4 to 6 cells was observed. However, the opposite trend was observed upon exposition to enhanced turbulence. In fact, the relative abundance of short chains (less than 3 cells) increased significantly with increasing CO_2 concentrations from 2 % at ~420 µatm to 98 % at ~2850 µatm. Furthermore, chains with more than 6 cells were only observed at CO_2 levels of ~420 and ~780 µatm decreasing from 83 to 22 %.

4 Discussion

4.1 Influence of CO_2 and turbulence on growth rates

At present day CO_2 concentrations, phytoplankton can be limited by the availability of dissolved inorganic carbon (Raven et al., 2014), meaning that diffusive CO_2 and (Miller et al., 1991; Rotatore et al., 1995; Li and Canvin, 1998; Burkhardt et al., 2001) and active uptake of CO_2 a and bicarbonate are not sufficient to support maximum photosynthetic rates (Riebesell et al., 1993; Morell et al., 2002). When exposed to enhanced turbulence, the effects of water motion near the cells might disrupt or eliminate the diffusive boundary layer, increasing CO_2 and HCO_3^- at the cells' surface at day time. As a consequence, the uptake of CO_2 and HCO_3^- are likely enhanced when compared to cells grown at more stable conditions. Cells exposed to enhanced turbulence could therefore, down-regulate the so-called carbon concentration mechanisms (CCM) saving energy, which potentially might be reallocated towards growth or other energy demanding processes. Here, it seems that *A. glacialis* utilized the excess energy to enhance growth and C, N and P production rates reaching values significantly higher than under more stable conditions. In a recent study which studied long term records of total diatom and dinoflaggelate abundanceds in the North Atlantic Subtropical Gyre, Barton et al. (2014) observed a prevalence of diatoms at more turbulent and dinoflagellates at more stable conditions.

At CO_2 concentrations of ~800 µatm, *A. glacialis* exposed to control conditions showed a peak in growth rate likely due to a compromise between increasing carbon availability and less favorable pH conditions. Stimulation of growth rate (Kim et al., 2006; Wu et al., 2010; Gao et al., 2012a; Yang and Gao, 2012; McCarthy et al., 2012; Barcelos e Ramos et al., 2014) and/or photosynthesis (Sun et al., 2011; Yang and Gao 2012; Gao et al., 2014) have been reported for some species of diatoms grown at similar CO_2 concentrations. Furthermore, recent investigations have suggested that doubling of ambient CO_2 concentrations could reduce the energy spent with CCM operation in several species of diatoms by up to 20 %, decreasing 3 to 6 % the total energy used for carbon fixation (Hopkinson et al., 2011). However, under enhanced turbulence, growth rates continuously decreased from ambient to increased CO_2 conditions (due to a shift of optimum growth towards lower CO_2 concentrations). This might be a consequence of higher energy demand for maintaining intracellular pH than energy saving with CCM operation under disrupted boundary layer (Berdalet and Estrada, 2005). Hence, higher turbulence acts to alleviate potential inorganic carbon limitation already at lower CO2 concentrations but exposes the cells to unfavorably low pH conditions earlier on.

At CO_2 concentrations higher than 1000 µatm, cell division rates of *A. glacialis* significantly decreased under control and enhanced turbulence conditions, even though more pronounced in the latter. This is most likely related to the decrease of sea water pH associated with the rise of CO_2 levels. In coccolithophores, intracellular pH regulation is mediated by voltage-gated H^+ channels (Hv channels) placed in the plasma membrane which dispose the excess protons (Taylor et al., 2012). At extracellular pH higher than 8.2, the H^+ efflux across the H_v channels occurs passively, making the process energetically feasible. However, at lower pH concentrations, the membrane potential changes and the H^+ efflux become an energy demanding processes. In order to guarantee cellular homeostasis, cells are forced to invest energy in the operation of the

Hv channels to cope with the external pH decrease. Indeed, acidified environments can affect the cells by compromising the diffusive boundary layer (Flynn et al., 2012), the intracellular enzyme and protein structure and activities (Beardall et al., 2009; Berge et al., 2010; Lu et al., 2011). Particularly, the activity of extracellular carbonic anhydrase has been seen to be inhibited by low pH levels (Aizawa and Miyachi 1986; Sultemeyer 1998; Bozzo and Colman, 2000; Gao et al., 2012b; Hopkinson et al., 2013).

4.2 Influence of enhanced CO_2 and turbulence on chain length and cell physiology

At control conditions, we observed a linear increase in chain length with rising CO_2 concentrations. Shorter chains (1 to 3 cells per chain) have thinner the boundary layers which decrease limitation of inorganic carbon near the cells (Barcelos e Ramos et al., 2014). Thus, cells in these chains could save energy by down-regulating the CCM. In agreement, Tchernov and Lipshultz (2008) found that in larger colonies of *Trichodesmium spp.* the diffusion of CO_2 into the cells is limited. When exposed to enhanced turbulence, the presence of longer chains of *A. glacialis* decreased as CO_2 concentrations were enhanced. A similar behavior has been previously observed in the diatoms *Chaetoceros spp.* and *Pseudo-nitzschia spp*. which showed longer chains in turbulent environment (Arin et al., 2002).

Increasing CO_2 levels triggered opposing trends under control and enhanced turbulence. The increase in chain length with enhanced CO_2, observed at stable conditions, here and in previous work (Barcelos e Ramos et al., 2014) might elevate the pH in the interior of the colonies and protect the cells from the acidified environment. However, in the case of *Skeletonema costatum* optimum chain length was associated with favorable growth conditions and high growth rates (Takabayashi et al., 2006). On the contrary, under enhanced turbulence condition and increased CO_2 levels, we observed a reduction in number of cells per chain associated with decreased growth rates, cellular elemental quotas and organic matter production. Reasoning for that might be related with the damaged and reduced boundary layer which cause an increase exposition of the cells to the acidified environment. Therefore, cells had to reallocate energy to regulate intracellular pH and cellular processes such as nutrient uptake or production of organic matter and extracellular polysaccharides which bond adjacent cells were compromised.

4.3 Particulate organic ratios

The bonds between cells of *A. glacialis* consist of mucilage polysaccharide pads associated with high C:N and C:P ratios (Beardall et al., 2009; Barcelos e Ramos et al., 2014). However, in agreement with previous studies conducted on *A. glacialis* (Barcelos e Ramos et al., 2014) and on *T. pseudonana* (McCarthy et al., 2012) and *E. huxleyi* (Borchard et al., 2011; McCarthy et al., 2012) in our investigations, no changes were observed in the C:N and C:P ratios across the experimental CO_2 range.

5 Conclusions

This study shows that turbulence can impact the CO_2 response of *A. glacialis*. At CO_2 concentrations comparable to present day levels, *A. glacialis* benefited from enhanced turbulence, with optimum rates shifted towards lower CO_2 concentrations. This could be related to a reduction of the diffusive boundary layer and the consequent increase of inorganic carbon availability near the cell. This could allow for down-regulating CCM operation and the energy saved could have been invested in others energy demanding processes. Under enhanced CO_2 levels, the costs of intracellular pH regulation outweigh the benefits of increased CO_2 concentrations. Thus, under enhanced turbulence and CO_2 concentrations, cells were forced to increase the energy investment in cellular homeostasis, while processes like growth rate, cellular quotas and organic matter production were reduced. Consequently, even though under present CO_2 conditions *A. glacialis* benefits from enhanced turbulence, under future ocean scenarios, it might be negatively affected with potential consequences for the phytoplankton community composition.

Author contributions:

F.G. and J.B.R. conceived and carried out the experimental work. K.G.S. carried out the POC and PON analyses. All authors contributed to the data analysis and to the preparation of the MS.

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Table 1. Carbonate chemistry parameters at the beginning, end and during the experiment (average).

Culture	Treatment	*p*CO_2 (µatm)	Avg *p*CO_2 (µatm)	TA (µmol kg^{-1})	pHt	HCO_3^- (µmol kg^{-1})	CO_3^{2-} (µmol kg^{-1})	CO_2 (µmol kg^{-1})	DIC (µmol kg^{-1})
Initial	1	512		2357	7.960	1946	164	16	2127
	2	1007		2355	7.701	2108	98	32	2238
	3	1435		2356	7.560	2171	73	46	2290
	4	3845		2362	7.154	2283	30	123	2467
Final Stable	1	330	421	2382	8.123	1823	224	11	2057
	1	328	420	2375	8.134	1816	224	11	2050
	1	325	419	2375	8.127	1813	225	10	2048
	1	308	410	2378	8.146	1797	233	10	2039
	2	544	775	2376	7.941	1977	160	17	2154
	2	521	764	2397	7.960	1980	167	17	2164
	2	545	776	2375	7.940	1976	159	17	2153
	2	484	746	2372	7.983	1940	172	16	2182
	3	825	1130	2366	7.781	2075	116	26	2217
	3	863	1149	2369	7.764	2087	112	28	2227
	3	789	1112	2367	7.798	2065	120	25	2211
	3	852	1144	2369	7.769	2084	113	27	2225
	4	1960	2903	2379	7.428	2235	57	63	2354
	4	1848	2846	2355	7.448	2206	59	59	2324
	4	1763	2804	2376	7.471	2219	62	63	2337
	4	1975	2909	2376	7.424	2231	56	59	2350
Final Turbulence	1	337	424	2378	8.115	1828	221	11	2059
	1	347	429	2362	8.102	1827	214	11	2052
	1	328	420	2372	8.124	1814	223	11	2048
	2	648	828	2365	7.873	2016	139	21	2176
	2	717	862	2370	7.836	2045	130	23	2198
	2	600	804	2369	7.903	1999	148	19	2166
	3	805	1120	2376	7.792	2077	119	26	2222
	3	782	1109	2375	7.803	2069	122	25	2216
	3	800	1118	2379	7.795	2078	120	26	2223
	4	1718	2782	2384	7.492	2222	64	55	2341
	4	1736	2790	2373	7.486	2214	63	56	2332
	4	1711	2778	2374	7.492	2212	64	55	2331

Figures

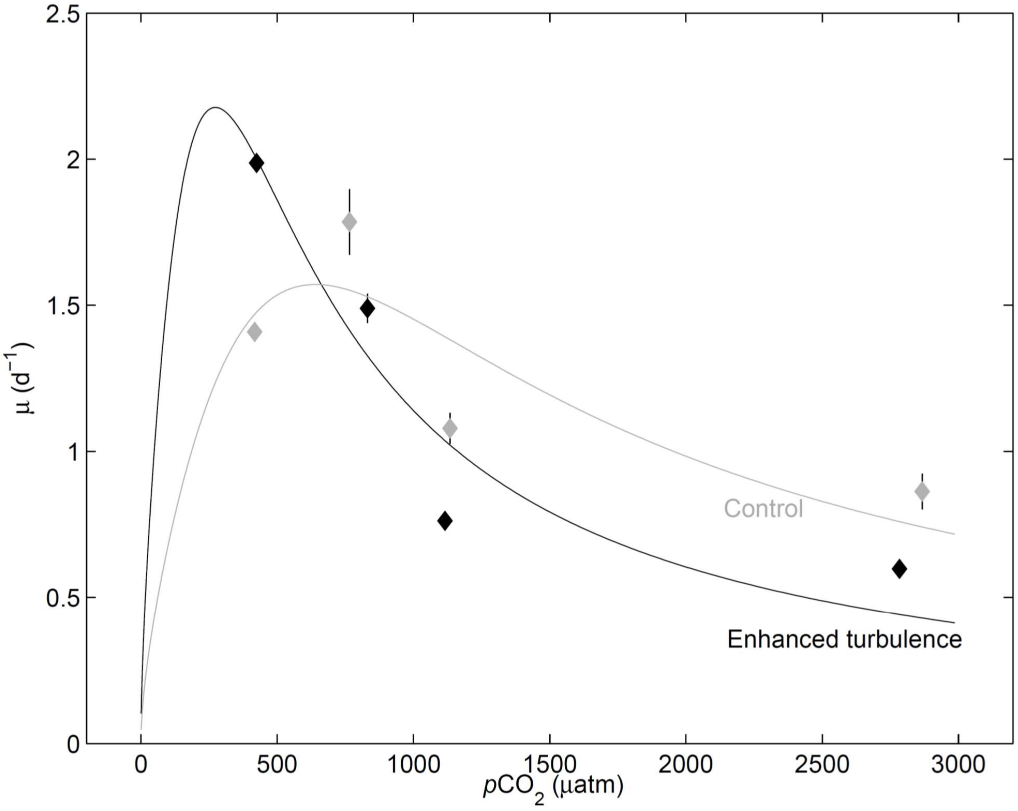

Figure 1. Growth rates of *Asterionellopsis glacialis* at increasing CO$_2$ levels (pCO$_2$) for the control and enhanced turbulence treatments. Growth rate curves were fitted to a CO$_2$/pH sensitive equation for growth (Gafar et al., to be submitted).

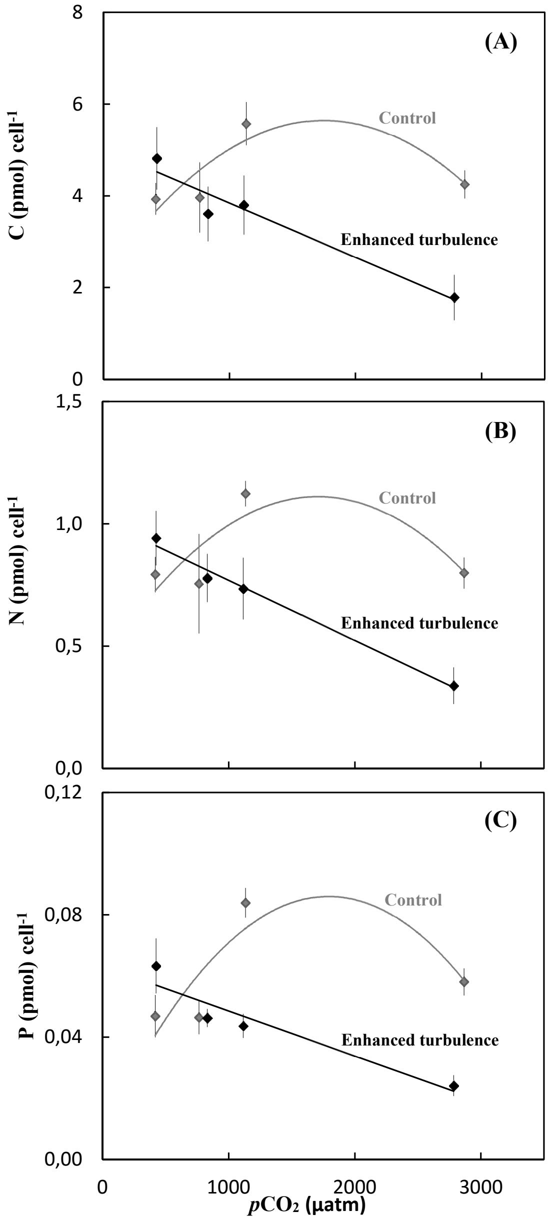

Figure 2. Cellular contents of *Asterionellopsis glacialis* at increasing CO_2 levels (pCO_2) under control and enhanced turbulence treatments (expressed as average and standard deviation). Carbon (A), nitrogen (B) and phosphorus (C). Polynomial fit of the data for control treatment: cellular POC: $y = -1E\text{-}06x^2 + 0.0039x + 2.2383$; PON: $y = -2E\text{-}07x^2 + 0.0008x + 0.437$; POP: $y = -2E\text{-}08x^2 + 9E\text{-}05x + 0.0088$. Linear fit of the data for enhanced turbulence treatment: cellular POC: $y = -0.0012x + 5.0232$ $R^2 = 0.9398$; PON: $y = -0.0002x + 1.0146$ $R^2 = 0.9894$; POP: $y = -1E\text{-}05x + 0.0633$ $R^2 = 0.902$.

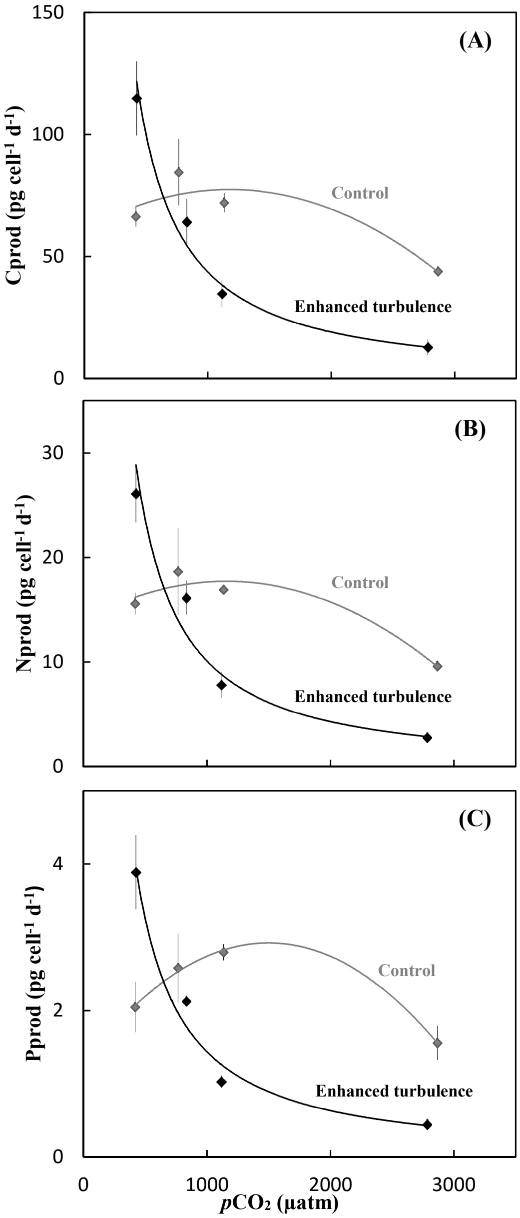

Figure 3. Organic matter production rates of *Asterionellopsis glacialis* at increasing CO_2 levels (pCO_2) under control and enhanced turbulence treatments (expressed as average and standard deviation). Carbon (A), nitrogen (B) and phosphorus (C). Polynomial fit of the data for control treatment: Cprod: $y = -1E\text{-}05x^2 + 0.0282x + 60.765$; Nprod: $y = -3E\text{-}06x^2 + 0.0065x + 13.977$; Pprod: $y = -7E\text{-}07x^2 + 0.0022x + 1.2818$. Polynomial fit of the data for enhanced turbulence treatment: Cprod: $y = 169420x^{-1.196}$ $R^2 = 0.9847$; Nprod: $y = 47922x^{-1.225}$ $R^2 = 0.97$; Pprod: $y = 4958.7x^{-1.18}$ $R^2 = 0.9715$.

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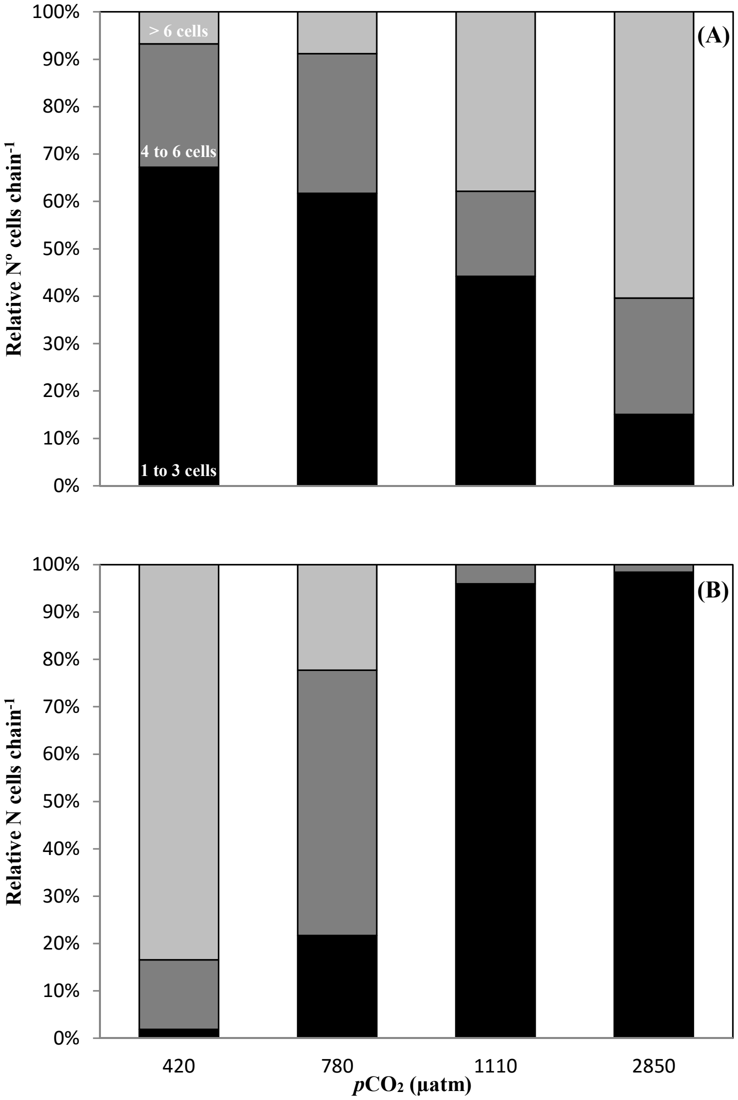

Figure 4. Relative number of cells per chain of *Asterionellopsis glacialis* at increasing CO_2 levels (*p*CO2) under stable (A) and turbulent environment (B).