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Carbon assimilation and losses during an ocean acidification mesocosm experiment, with special reference to algal blooms

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ABSTRACT

A mesocosm experiment was conducted in Wuyuan Bay (Xiamen), China, to investigate the effects of elevated pCO_2 on bloom formation by phytoplankton species previously studied in laboratory-based ocean acidification experiments, to determine if the indoor-grown species performed similarly in mesocosms under more realistic environmental conditions. We measured biomass, primary productivity and particulate organic carbon (POC) as well as particulate organic nitrogen (PON). *Phaeodactylum tricornutum* outcompeted *Thalassiosira weissflogii* and *Emiliania huxleyi*, comprising more than 99% of the final biomass. Mainly through a capacity to tolerate nutrient-limited situations, *P. tricornutum* showed a powerful sustained presence during the plateau phase of growth. Significant differences between high and low CO_2 treatments were found in cell concentration, cumulative primary productivity and POC in the plateau phase but not during the exponential phase of growth. Compared to the low pCO_2 (LC) treatment, POC increased by 45.8–101.9% in the high pCO_2 (HC) treated cells during the bloom period. Furthermore, respiratory carbon losses of gross primary productivity were found to comprise 39–64% for the LC and 31–41% for the HC mesocosms (daytime C fixation) in phase II. Our results suggest that the duration and characteristics of a diatom bloom can be affected by elevated pCO_2. Effects of elevated pCO_2 observed in the laboratory cannot be reliably extrapolated to large scale mesocosms with multiple influencing factors, especially during intense algal blooms.

1. Introduction

As a result of human activity, anthropogenic emissions of CO_2 have been increasing from the pre-industrial 280 ppmv to the present-day value of about 400 ppmv, and these will further increase to 800–1000 ppmv by the end of this century according to the Intergovernmental Panel on Climate Change (Pachauri et al., 2014). As a major sink, the ocean has absorbed approximately 30% of anthropologically derived CO_2, leading to ocean acidification (OA), with ocean pH expected to decrease by 0.3–0.4 units by the end of this century (Rhein et al., 2013; Pachauri et al., 2014).

As CO_2 is the substrate of photosynthesis, alterations to the concentration of $[CO_2]_{aq}$ in seawater are known to have significant effects on marine primary producers (Gao et al., 2012; Mostofa et al., 2016). Generally, it is thought that the enhancement of $[CO_2]_{aq}$ will reduce the energy consumption through down-regulation of carbon dioxide concentrating mechanisms (CCMs), and that the saved energy might enhance the growth of phytoplankton (Hopkinson et al., 2011). However, the increase of pCO_2 will be accompanied by a pH decline, which will cause cells to consume more energy to maintain a constant intracellular pH (Suffrian et al., 2011; Bach et al., 2013). Therefore, the impacts of elevated pCO_2 on phytoplankton are complicated in view of the changes in both substrates for photosynthetic and carbonate chemistry.

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To date, many studies have examined the influence of elevated pCO_2 on marine primary producers, especially diatoms and coccolithophores. Diatoms are important marine primary producers as they contribute nearly half of oceanic productivity, so their responses to elevated pCO_2 are of great significance. Elevated CO_2 associated with decreased pH may result in different responses (positive, neutral and negative effects) in various diatom species (see review by Gao and Campbell, 2014 and literature cited therein). These different results may stem from different experimental designs, such as levels of light and temperature, as well as different species-specific physiology. Coccolithophores are another important phytoplankton group, performing calcification in addition to photosynthetic carbon fixation. The relative capacities of photosynthesis and calcification have considerable influence on the pH around cells and subsequently on global biogeochemical cycles (Zondervan et al., 2002; De Bodt et al., 2010). However, reported results for the effects of elevated pCO_2 on the photosynthetic process of coccolithophores are not consistent (Orr et al., 2005; Langer et al., 2006). Both coccolithophores and diatoms are widespread in the world oceans, and coccolithophore blooms are often observed to occur after those of diatoms (Heimdal et al., 1994; Brown and Yoder, 1994; Brown, 1995). The amounts of fixed carbon and assimilated nitrogen by diatoms and coccolithophores are of general concern regarding future oceanic carbon sequestration and global biogeochemical C and N cycles, since primary production mediates the transformation of CO_2 into organic carbon with variable stoichiometric relationships to other major elements, such as nitrogen (N) and phosphorus (P) (Engel et al., 2013). Due to the presence of the coccoliths in coccolithophores, the ratio of diatoms relative to coccolithophores influences the ratio of organic relative to inorganic carbon in sedimenting particles, which is important for deep-sea carbon storage.

Many studies related to the effects of elevated pCO_2 on phytoplankton have been carried out in small scale laboratory experiments, usually in cultures of less than a litre, maintained under constant light and temperature levels. Although these studies are very important to reveal mechanistic responses to changing seawater carbonate chemistry caused by ocean acidification, the results are difficult to extrapolate to natural dynamic environments. Mesocosms are known to provide a powerful tool to maintain a relatively complex community, which take relevant aspects from “the real world” into account (Riebesell et al., 2013).

Here, we report on a mesocosm experiment conducted to study the influence of elevated pCO_2 on the primary production and biogeochemical cycle of an artificial phytoplankton community, including diatoms and coccolithophores, which had been grown in the laboratory and had been previously examined for their responses to elevated pCO_2. We hypothesized that the effects of elevated pCO_2 on the diatoms and coccolithophores obtained in the laboratory might differ when these species are grown at a large scale under the influence of multiple factors in the sea.

2. Materials and methods

2.1. Experimental setup

The mesocosm experiments were carried out on a floating platform at the Facility for Ocean Acidification Impacts Study of XMU (FOANIC-XMU, 24.52°N, 117.18°E) in Wu Yuan Bay between 22 December 2014 and 24 January 2015 (the day for algae inoculation was set as day 0). Six cylindrical transparent thermoplastic polyurethane (TPU) bags with domes were deployed along the south side of the platform. The width and depth of each mesocosm bag was 1.5 m and 3 m, respectively.

Filtered (0.01 μm, achieved using an ultrafiltration water purifier, MU801-4T, Midea, China) *in situ* seawater was pumped into the six bags simultaneously within 24 h. A known amount of NaCl solution was added into each bag to calculate the exact volume of seawater in the bags, according to the comparison of the salinity before and after salt addition (Czerny et al., 2013). The initial *in situ* pCO_2 was about 650 μatm. To set the low and high pCO_2 levels, we added Na_2CO_3 solution and CO_2 saturated seawater into mesocosm bags to alter the TA (total alkalinity) and DIC (dissolved inorganic carbon) (Gattuso et al., 2010; Riebesell et al., 2013). Subsequently, during the whole experimental process, air at the ambient (400 μatm) and elevated pCO_2 (1000 μatm) concentrations were continuously bubbled into the mesocosm bags using a CO_2 Enricher (CE-100B, Wuhan Ruihua Instrument & Equipment Ltd, China). A flow rate of about 5 L per minute was applied for each bag, and the air was dispersed at the bag's bottom using pre-cleaned airstones.

2.2. Algal strains

Three phytoplankton strains were inoculated into the mesocosm bags, all species at 4×10^4 cells L^{-1}. Both *Phaeodactylum tricornutum* (CCMA 106) and *Thalassiosira weissflogii* (CCMA 102) were obtained from the Center for Collections of Marine Bacteria and Phytoplankton (CCMBP) of the State Key Laboratory of Marine Environmental Science (Xiamen University), the former being originally isolated from the South China Sea (SCS) in 2004 and the other isolated from Daya Bay in the coastal South China Sea. *Emiliania huxleyi* PML B92/11, was originally isolated in 1992 from the field station of the University of Bergen (Raunefjorden; 60°18′N, 05°15′E).

2.3. Measurements of chlorophyll a

Chlorophyll *a* (Chl *a*) was measured using water samples (200 mL-1000 mL) collected every two days at 9 a.m. by filtering onto Whatman GF/F filters (diameter: 25 mm; pore diameter: 0.7 μm). The filters were placed into 5 mL 100% methanol overnight at 4 °C and centrifuged at 5000 g for 10 min. The absorbance of the supernatant (2.5 mL) was measured from 250 to 800 nm using a scanning spectrophotometer (DU 800, Beckman Coulter Inc).

2.4. Primary productivity

The measurement of primary productivity was conducted every two days. Just before sunrise, an 80 mL sample was withdrawn from each bag using a syringe, and divided into four glass scintillation vials for different treatments.

100 μL $NaH^{14}CO_3$ (ICN Radiochemicals, Irvine, CA, USA) containing 5 μCi (0.185 MBq) ^{14}C was added to each glass scintillation vial (20 mL). Vials, covered with a dark neutral net to provide similar light levels as in the bags, were incubated under sunlight with temperature controlled by flowing *in situ* seawater. Over the following 24 h, samples were filtered onto GF/F glass filters every 12 h, and the filters stored at −20 °C. After fuming overnight with concentrated HCl, the membrane samples were dried (50 °C, 6 h) to expel non-fixed labeled carbon (Gao et al., 2007). 4 mL of scintillation cocktail (Hisafe 3, Perkin-Elmer) was added to each vial, then a liquid scintillation counter (Tri-Carb 2800TR, Perkin-Elmer, Waltham, USA) was used to count the radioactivity of fixed ^{14}C.

2.5. C and N measurements

Samples for particulate organic carbon (POC) and particulate organic nitrogen (PON) determination were taken at the same time as those for Chl *a*. Water samples were filtered onto pre-combusted