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Inorganic carbon availability in benthic diatom communities: photosynthesis and migration

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Diatom-dominated microphytobenthos (MPB) is the main primary producer of many intertidal and shallow subtidal environments, being therefore of critical importance to estuarine and coastal food webs. Owing to tidal cycles, intertidal MPB diatoms are subjected to environmental conditions far more variable than the ones experienced by pelagic diatoms (e.g. light, temperature, salinity, desiccation and nutrient availability). Nevertheless, benthic diatoms evolved adaptation mechanisms to these harsh conditions, including the capacity to move within steep physical and chemical gradients, allowing them to perform photosynthesis efficiently. In this contribution, we will review present knowledge on the effects of dissolved inorganic carbon (DIC) availability on photosynthesis and productivity of diatom-dominated MPB. We present evidence of carbon limitation of photosynthesis in benthic diatom mats and highly productive MPB natural communities. Furthermore, we hypothesize that active vertical migration of epipelic motile diatoms could overcome local depletion of DIC in the photic layer, providing the cells alternately with light and inorganic carbon supply. The few available longer-term experiments on the effects of inorganic carbon enrichment on the productivity of diatom-dominated MPB have yielded inconsistent results. Therefore, further studies are needed to properly assess the response of MPB communities to increased CO_2 and ocean acidification related to climate change.

This article is part of the themed issue 'The peculiar carbon metabolism in diatoms'.

1. Introduction

Microphytobenthos (MPB) are phototropic communities of intertidal and shallow subtidal ecosystems that are largely dominated by diatom species (figure 1). MPB are responsible for a significant fraction of the total primary productivity of estuaries and coastal zones, mediating nutrient cycling and efficiently stabilizing the sediment [1,2], both processes being largely mediated by the excretion of carbohydrate-enriched extracellular polymeric substances by diatom cells [3,4]. The microenvironment of these densely populated MPB communities is complex and highly heterogeneous, characterized by steep gradients in physical and chemical parameters such as light, temperature, salinity and nutrient availability. A major determining factor of MPB abundance and species composition is the nature of the substratum, with fine cohesive sediments supporting significantly higher MPB biomass and an increasing proportion of epipelic motile taxa when compared with sandier sediments. The exact mechanisms driving diatom motility have been the subject of considerable speculation, although it has been established that it involves secretion of mucilage that promotes cell adhesion to the substrate [5]. In the case of raphid diatoms, mucilage secretion occurs through the raphe fissure and motility involves a complex mechanism powered by a membrane-associated

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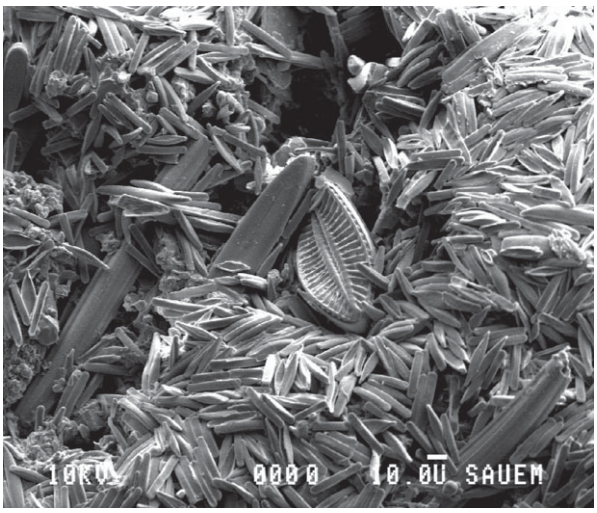

Figure 1. Low-temperature scanning electron micrograph of the surface of an intertidal cohesive sediment from the Tagus estuary (Portugal), during low tide exposure, showing an extremely dense biofilm of motile pennate diatoms.

microfilamentous system [6]. The ecological success of diatoms in intertidal MPB communities has frequently been linked to cell motility as it allows cells to search for optimal environmental conditions with regards to decisive abiotic parameters, namely light intensity (e.g. [7,8]).

2. Carbon limitation in benthic diatom communities

Despite the fact that concentrations of dissolved inorganic carbon (DIC) in seawater are usually high and relatively constant (approx. 2 mmol l^{-1}), the low diffusion coefficient for CO_2 in water has the potential to restrict the rate of photosynthesis in diatoms [9]. CO_2 is the inorganic carbon form used by the ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco; EC 4.1.1.39), which only accounts for approximately 1% of total inorganic carbon, HCO_3^- being the predominant form. Therefore, the usual CO_2 concentration in seawater (at atmospheric equilibrium at 25°C) is around 10 μM. As the reported $K_{1/2}$ (CO_2) of diatoms' Rubisco is 23–68 μM [10], photosynthesis of marine diatoms could, in principle, be limited by carbon supply [11]. In fact, in spite of the shared lineage of their chloroplasts, diatoms' Rubisco presents a much lower affinity for CO_2 than that from red algae, and even lower than the one from vascular plants. Only cyanobacterial Rubisco shows an affinity for CO_2 lower than that of diatoms [12,13]. Nevertheless, the general belief of CO_2-insensitive photosynthesis in diatoms has prevailed due to the presence of highly efficient CO_2-concentrating mechanisms (CCMs) [14–17]. These are so outstanding that it is plausible to attribute, in large part, today's atmospheric CO_2 concentration to the efficiency of the CCMs of diatoms [18].

Carbon concentrating mechanisms may be divided into two categories: biophysical and biochemical mechanisms [19]. The former is based on the operation of membrane transporters specific for inorganic carbon forms, conjugated with the activity of the enzyme carbonic anhydrase (CA; EC 4.2.1.1), which catalyses the interconversion of carbonic acid and carbon dioxide. Most carbonic anhydrases of diatoms are zinc metalloenzymes, but a unique CA was found with a prosthetic group where the bound metal is cadmium [20]. Biochemical mechanisms are based on the operation of β-carboxylases, namely phosphoenolpyruvate carboxylase (PEPC; EC 4.1.1.31). The photosynthetic role of PEPC has been long established in higher plants [21], but in diatoms it is still speculative [22]. Biological carbon isotope discrimination is dependent both on physical constraints (e.g. diffusion pathways) and biochemical constraints (e.g. carboxylating enzyme activity), reflecting, therefore, the activity of CCMs. In marine environments, it is usually assumed that the biochemical contribution to carbon isotope discrimination is constant and equal to the fractionation due to the carboxylation by Rubisco [23]. However, PEPC activity, which has a different discrimination factor, could contribute to the overall carbon discrimination observed in marine environments. There are two different putative roles attributed to PEPC (and another β-carboxylase, phosphoenolpyruvate carboxykinase (PEPCK; EC 4.1.1.49)) in diatom metabolism: one is a CCM acting upstream and in-line with Rubisco (i.e. a C_4-like pathway); the other is an anaplerotic role, feeding the Krebs cycle with oxaloacetate resulting from the carboxylation of phosphoenolpyruvate. Owing to various confounding factors (e.g. cell surface area to volume ratio [24]) the relative contribution of Rubisco and β-carboxylases is difficult to decipher. In a controlled experiment with the model diatom species *Phaeodactylum tricornutum*, Cassar & Laws [23] showed that the metabolic role of β-carboxylases is anaplerotic and has a marginal impact on the carbon fractionation.

Regardless of the mechanistic basis, it is well established that CCMs increase the CO_2 concentration and the CO_2/O_2 ratio at the Rubisco catalytic sites, increasing its carboxylation specific activity. This is achieved most probably by an increased CO_2 concentration within the pyrenoid, a low CO_2-permeable organelle of diatom chloroplasts where Rubisco is located [18]. The low permeability of the pyrenoid to CO_2 is probably due to its proteic nature, since lipid membranes are extremely permeable to small neutral molecules, such as CO_2, in contrast with charged ions such as HCO_3^- [18]. Hopkinson *et al.* [18] found values of membrane permeability to CO_2 ranging from 1.5×10^{-2} to 5.6×10^{-2} cm s^{-1} in four diatom species, making clear that the plasmalemma is not a significant barrier to CO_2. Therefore, a DIC transport system based on membrane CO_2 transporters would be extremely inefficient. By contrast, the values of membrane permeability to HCO_3^- were several orders of magnitude lower, making its passive transport negligible. In any case, the free diffusion of CO_2 across the plasmalemma and the chloroplast envelope is unable to increase the CO_2 concentration at the carboxylation sites of Rubisco above the external environmental levels. A CCM transporting either HCO_3^- or a C_4 compound is required [18]. These authors, however, estimated that only one-third of the carbon fixed by the diatom *P. tricornutum* is actively transported, the remaining reaching the chloroplast by CO_2 diffusion. Owing to the efficiency of CCMs, diatoms allocate their scarce resources conservatively to Rubisco [10], exhibiting a much lower content than higher plants [25,26]. However, significant differences were found between species, with diatoms such as *Thalassiosira* and *Skeletonema* exhibiting low Rubisco content but demanding higher resource allocation to their CCMs in order to saturate their low CO_2-affinity Rubisco, and species such as *Phaeodactylum* and *Chaetoceros* showing high Rubisco

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Table 1. Studies on the effects of increased inorganic carbon availability on diatom-dominated MPB natural communities. n.d., not determined.

study	increased carbon availability	time frame	effects on MPB photosynthesis/biomass	effects on community composition
35	increased flushing; addition of HCO_3^-	3 h	+	n.d.
36	addition of HCO_3^-	30 min	+	n.d.
38	natural CO_2 gradient	21 days	+	increased growth of large pennates
39	water acidification by CO_2 bubbling	11 days	+ (− under increased T°C)	no effects detected (abundance changes under increased T°C)
40	increased atmospheric CO_2	7 days	no effects detected (− under increased T°C)	n.d.

content, with more affinity for CO_2, requiring lower allocation of resources to their CCMs. Therefore, an inverse relation between the amount of cellular Rubisco and its K_m for CO_2 was found [10]. Rubisco is often considered an inefficient catalyst because, when compared with other photosynthetic enzymes, it exhibits a low maximal (i.e. CO_2-saturated) CO_2 fixation rate and elevated Michaelis–Menten constant for CO_2 [10]. The complex catalytic mechanism of Rubisco imposes trade-offs between the CO_2-saturated carboxylation rate, the affinity for CO_2 and the relative specificity for CO_2 [27,28]. Since CCMs grant full saturation of Rubisco catalytic sites, it is believed that the photosynthesis of pelagic diatoms is not limited by DIC [29,30], although some studies suggest that such a possibility could occur [7]. In fact, Hopkinson *et al.* [18] estimated that the CCMs, albeit significantly increasing the CO_2 concentration in the chloroplasts above ambient levels, are not able to saturate the carboxylation sites of Rubisco. They projected that the average concentration of CO_2 in the chloroplast is 15–20 μM, whereas the half-saturation constant of *P. tricornutum*'s Rubisco to CO_2 is 40 μM [11]. A higher CO_2 concentration may be achieved in the pyrenoids, where most of the Rubisco is located [18]. Since there seems to be little excess capacity of Rubisco in relation to photosynthesis, the enzyme must operate at full saturation [18]. After being converted to CO_2 by CA, roughly two-thirds of the DIC transported to the chloroplast diffuses back to the cytoplasm, whereas nearly one-third is fixed by Rubisco. The leaked CO_2 is hydrated to HCO_3^- in a reaction catalysed by a cytoplasmic CA. The activity of this enzyme is an important element of the diatom CCM, allowing passive influx of CO_2 from the external medium and recycling most of the CO_2 leaking from the chloroplast [18].

CCMs are also expected to occur in benthic diatoms. According to several authors (e.g. [31]), CCMs are ubiquitous among long-lasting low-CO_2 microhabitats, including MPB. In the latter environments, the high concentration of photosynthetic cells together with the low diffusion rate from the bulk medium to the cells, due to thick diffusion boundary layers, may create DIC limitation to photosynthesis. In these situations, extreme conditions may occur inside the photosynthesising layer, such as oxygen partial pressure over 1 mbar and pH values above 9 due to photosynthetic activity [32,33]. A number of studies on diatom-dominated benthic communities have challenged the notion of CO_2-insensitive photosynthesis in diatoms and suggested limitation by inorganic carbon availability. Admiraal *et al.* [34] found that the diffusion of inorganic carbon limited the productivity of dense unialgal mats of the benthic diatom *Navicula salinarum*. Addition of inorganic carbon was found to increase photosynthetic rates of MPB natural communities dominated by diatoms in subtidal sand and in intertidal muddy sediments ([35,36]; table 1). Cook & Roy [35] showed that increasing the flux of water (and therefore of the DIC) through the sediment increased the rate of photosynthesis (measured by the incorporation of $^{14}CO_2$) from 6.4 to 8.6 mmol m^{-2} h^{-1}, showing that a limitation of photosynthesis by DIC availability is present. Whereas flushing did not alter the depth-specific maximum rates of photosynthesis, the integrated rates of photosynthesis were higher due to the widening of the photosynthetic zone. These results suggest that pore-water transport can enhance photosynthesis by counteracting DIC limitation in permeable sediments. After the addition of inorganic carbon, we observed increased photosynthetic electron transport rates in two intact MPB communities of intertidal cohesive sediments (Alcochete and Trancão) differing in DIC availability (figure 2). Inorganic carbon was added in the form of $NaHCO_3$ solution to the sediment surface after biofilm establishment and photosynthetic activity was assessed after 30 min using an imaging pulse amplitude modulated (PAM) fluorometer. Vieira *et al.* [36], based on carbon isotope discrimination data of these two different MPB communities, suggested that benthic diatoms can reduce the energy costs associated with their CCMs under increasing inorganic carbon availability.

Longer-term experiments of inorganic carbon enrichment have yielded inconsistent results on photosynthesis, growth and composition of MPB communities and cultures of benthic diatoms. Examining the colonization of artificial substrata across a natural CO_2 gradient, Johnson *et al.* [38] found that elevated CO_2 increased MPB biomass and induced diatom community shifts by promoting the growth of large pennate species. In an 11-day microcosms experiment, Cartaxana *et al.* [39] reported a beneficial effect of elevated CO_2 on MPB biomass of a muddy intertidal sediment community dominated by *Navicula spartinetensis* and *Gyrosigma acuminatum*, but no significant effects on the relative abundance of major groups of microalgae and benthic diatom species. However, the interactive effect of increased CO_2 and temperature had a detrimental effect on MPB biomass and led to an increase of cyanobacteria together with a change in the relative abundance of major diatom species.

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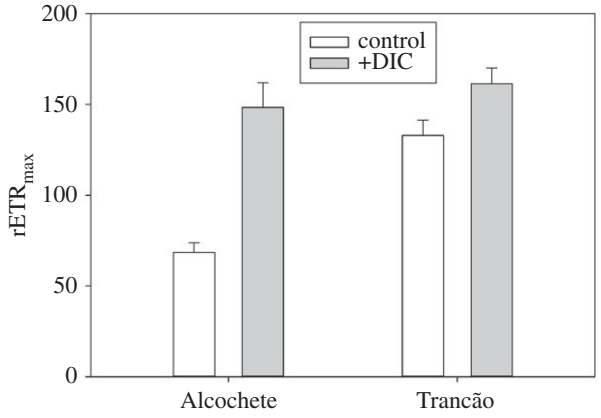

Figure 2. Maximum relative electron transport rates ($rETR_{max}$) of photosynthesis (mean $\pm$ s.d., $n = 5$) for control and carbon-enriched intertidal sediments of the Tagus estuary (Portugal) colonized by two benthic diatom communities (Alcochete and Trancão). Measurements were obtained using rapid light curves (RLC) performed with an imaging PAM fluorometer on 2 cm diameter sediment samples [37]. Control, addition of 150 µl of seawater; +DIC, addition of 150 µl of 50 mM $NaHCO_3$ solution.

In a 7-day experiment, Hicks *et al.* [40] found no significant increase in MPB biomass in a muddy intertidal sediment under increased CO_2 levels, but observed a detrimental effect under increased temperature (table 1). Torstensson *et al.* [41] reported that the benthic diatom *Navicula directa* cultured in liquid medium was negatively affected by CO_2 enrichment, although the mechanism causing this effect was not identified. Similarly, a small reduction in growth and increased dissolved organic carbon exudation was observed in cultures of the sea ice diatom *Nitzschia lecointei* after 147 days at elevated CO_2 conditions [42]. The latter study illustrates the importance of experimental duration and shows that more studies are needed to properly assess the impact of climate change and understand the effects of long-term increased CO_2 and ocean acidification on MPB communities.

3. Light, inorganic carbon availability and diatom migration

Motile diatoms, referred to as epipelic, are dominant in intertidal muddy sediments and exhibit migratory rhythms synchronized with diurnal and tidal cycles [43,44]. These MPB cells accumulate at the surface of the sediment during diurnal low tides, forming dense, highly productive biofilms and migrate downwards before tidal inundation or darkness. Several studies have reported the endogenous nature of this behaviour, showing that the vertical movements of epipelic diatoms continue for several days in the absence of external stimuli (e.g. [45]). Upward migration during diurnal exposure allows cells to reach the photic zone, causing significant changes in productive biomass [46]. The depth of the sediment in which benthic diatom cells migrate is affected by many factors, including sediment type, depth of light penetration, dominant taxa present and speed of cell locomotion [47], but it is generally limited to the uppermost millimetre [48].

Motility is an adaptation of the upmost importance considering the extremely steep light gradient found in cohesive sediments (figure 3). Light available for photosynthesis is increased at the sediment surface due to a strong component

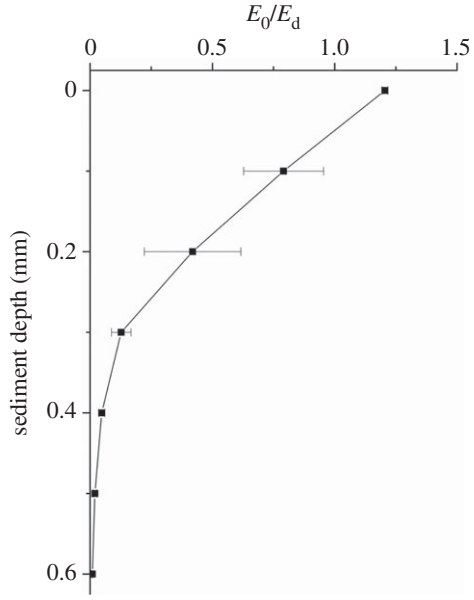

Figure 3. Photon scalar irradiance (E_0) depth microprofile of an intertidal muddy sediment of the Tagus estuary (Portugal) normalized to the downwelling photon irradiance at the surface (E_d). Average $\pm$ s.d. of three incident downwelling photon irradiances: 250, 500 and 1000 µmol photons m^{-2} s^{-1}. Measurements were obtained with a scalar irradiance microprobe [49] consisting of a small diffusing sphere (80 µm diameter) cast on the coated tip of a tapered optical fibre.

of scattered light. However, photon scalar irradiance is strongly attenuated with sediment depth, reaching less than 1% of incident downwelling irradiance below 0.5 mm (figure 3). Furthermore, strongest light attenuation is observed for wavelengths more relevant for photosynthesis, corresponding to absorption maxima of diatom photopigments (chlorophylls and carotenoids), thus limiting photosynthetic activity in sediment layers very close to the surface [50].

In addition to the above described vertical bulk migration, epipelic diatoms can respond to changes in light levels and migrate downwards in the sediment when exposed to very high irradiances [7,51]. High light mainly causes reallocation of cells within the photic zone to more optimal light conditions and not necessarily massive bulk migration of MPB to deeper aphotic layers. According to Kromkamp *et al.* [52], motile MPB cells are continuously replaced by others at the sediment surface, thus avoiding photoinhibition, a mechanism the authors termed 'micromigration' (figure 4). Subsequent studies have confirmed the importance of this photoregulatory strategy in epipelic MPB communities of cohesive intertidal sediments, where cells experience significant changes in photon irradiance by covering submillimetre distances in a highly heterogeneous light environment (e.g. [8]). On the contrary, non-motile epipsammic diatom species of sandier substrata rely exclusively on physiological photoprotection mechanisms [51,53], as also do pelagic diatoms. Energy dissipation mechanisms in diatoms are more efficient than in higher plants, as highlighted by the three to five times larger non-photochemical chlorophyll quenching measured in the model diatom *P. tricornutum* [54,55], when comparing with *Spinacia oleracea* and *Arabidopsis thaliana* counterparts [55]. Using unialgal cultures, Barnett *et al.* [56] described the existence of a trade-off between behavioural (motility) and physiological photoprotective mechanisms (non-photochemical-quenching and the

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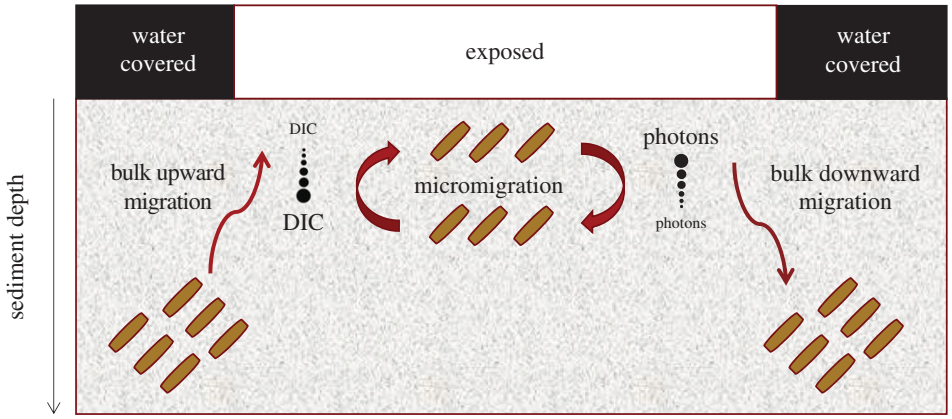

Figure 4. Schematic of benthic intertidal diatom migration, light and dissolved inorganic carbon (DIC) depth-gradients during diurnal exposure in intertidal cohesive sediments. (Online version in colour.)

xanthophyll cycle) in epilelic and epipsammic benthic diatom growth forms.

None of the studies reporting downwards migration of epipelic diatoms in response to high light assessed associated changes in the availability of inorganic carbon in the photic layer, so the putative role of CO_2 availability in diatom micromigration is unclear. In sediments, DIC measurements have mostly been determined by bulk pore-water analysis at the centimetre and millimetre scales. However, in sediments with thin photic layers (a few hundreds of micrometre in thickness) such pore-water analyses have insufficient spatial resolution. Vieira *et al*. [36] found net photosynthetic rate of MPB suspensions to reach saturation at a DIC concentration significantly lower than the concentrations found in the interstitial water of the top 5-mm sediment layer. However, these authors observed that the addition of HCO_3^- to intact sediments increased the photosynthetic capacity of the MPB and concluded that the DIC measured in the 5-mm layer was not relevant for the phototrophic community. To the contrary, DIC was depleted in the thin photic layer caused by massive migration of diatom cells to the sediment surface ([36]; figures 1 and 4). *In situ* determination with sensors with thin tips of only a few micrometres can circumvent this problem and produce measurements at the relevant spatial scale [57]. Nevertheless, high-spatial resolution DIC profiles obtained with microsensors require simultaneous paired measurements of CO_2 and pH that are not easily obtainable. Furthermore, reliable DIC estimates require the assumption that the inorganic carbon system is at equilibrium, which is difficult to establish. Despite these methodological limitations, submillimetre depth distributions of DIC derived from pH and CO_2 profiles measured with microelectrodes in organic-rich sediments reveal a strong vertical gradient with DIC depletion at the surface and increasing concentrations with depth ([58]; figure 4). Therefore, micromigration alternates the exposure of diatom cells between surface microenvironments with high light and low DIC availability and subsurface microenvironments with little or no light and high DIC availability, creating a putative time-delay between the production of energy and reductants and the reduction of inorganic carbon, resembling the photosynthetic crassulacean acid metabolism of xerophytic higher plants.

4. Conclusion

In intertidal sediments, benthic epipelic diatom cells accumulate at the sediment surface at diurnal low tide with most of the biomass concentrated in the first hundreds of micrometres. During this period, photosynthetic activity of MPB is extremely high, causing local depletion of DIC in the photic layer. We hypothesize that micromigration of diatoms within the uppermost layers of the sediment overcomes this limitation for photosynthesis, providing the cells alternately with light and inorganic carbon supply, while reducing photoinhibition. The few available longer-term experiments on the effects of inorganic carbon enrichment on the productivity and composition of diatom-dominated MPB communities have provided inconsistent results. Hence, further studies are needed to understand the longer-term response of MPB to increased CO_2 and ocean acidification related to climate change.

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Competing interests. We have no competing interests.

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