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REVIEW PAPER

# The possible evolution and future of $CO_2$-concentrating mechanisms

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## Highlight

The probable timing of the origin of CCMs in cyanobacteria and eukaryotic algae is computed from Rubisco kinetics and $CO_2$ changes over geological time. An analysis of factors determining the role of CCMs with global environmental change is presented.

## Abstract

$CO_2$-concentrating mechanisms (CCMs), based either on active transport of inorganic carbon (biophysical CCMs) or on biochemistry involving supplementary carbon fixation into $C_4$ acids ($C_4$ and CAM), play a major role in global primary productivity. However, the ubiquitous $CO_2$-fixing enzyme in autotrophs, Rubisco, evolved at a time when atmospheric $CO_2$ levels were very much higher than today and $O_2$ was very low and, as $CO_2$ and $O_2$ approached (by no means monotonically), today's levels, at some time subsequently many organisms evolved a CCM that increased the supply of $CO_2$ and decreased Rubisco oxygenase activity. Given that $CO_2$ levels and other environmental factors have altered considerably between when autotrophs evolved and the present day, and are predicted to continue to change into the future, we here examine the drivers for, and possible timing of, evolution of CCMs. CCMs probably evolved when $CO_2$ fell to 2–16 times the present atmospheric level, depending on Rubisco kinetics. We also assess the effects of other key environmental factors such as temperature and nutrient levels on CCM activity and examine the evidence for evolutionary changes in CCM activity and related cellular processes as well as limitations on continuity of CCMs through environmental variations.

**Key words:** Algae, cyanobacteria, $CO_2$-concentrating mechanisms, $CO_2$ diffusion, evolution, Rubisco.

## Introduction

All cyanobacteria, eukaryotic algae, and embryophytes ('plants') rely on the enzyme ribulose-1,5-bisphosphate carboxylase oxygenase (Rubisco) and the photosynthetic carbon reduction cycle (PCRC; otherwise known as the Calvin–Benson–Bassham cycle) for the net assimilation of inorganic carbon to organic matter. Less than 1% of the

primary production on the planet is carried out by processes that do not involve Rubisco (Raven, 2009; Beardall and Raven, 2016)

There is a broad range of Rubisco forms with differing kinetic properties (Beardall and Raven, 2016). However, even the Rubiscos with the highest $CO_2$ affinity are not saturated by dissolved $CO_2$ in equilibrium with present atmospheric $CO_2$ concentrations at the temperatures at which the organisms from which the Rubiscos were derived normally grow (Uemura et al., 1997; Galmés et al., 2016). All Rubiscos also have a competing oxygenase reaction that uses a photorespiratory carbon oxidation cycle (PCOC) or its equivalent to deal with the 2-phosphoglycolate produced by the oxygenase reaction. This increases the energy and other resource costs of photosynthesis in today's atmosphere with diffusive $CO_2$ entry into cells, relative to when Rubisco is operating at $CO_2$ saturation. Even organisms with relatively high $CO_2$ affinity Rubiscos sometimes have CCMs involving biophysical processes based on active transport of inorganic carbon species (cyanobacteria and eukaryotic algae) or biochemical pathways based on additional, high affinity, carboxylases [C$_4$ and CAM (Crassulacean acid metabolism) plants] (see Beardall and Raven, 2016, and references therein). These CCMs have their own energy and other resource costs, and the factors determining the relative selective advantages of diffusive $CO_2$ entry and of CCMs are incompletely understood, except that organisms possessing Rubiscos with very low $CO_2$ affinity (cyanobacteria and basal dinoflagellates) always have CCMs (e.g. Raven and Beardall, 2014, 2016*a*, *b*).

There are few exceptions (Raven et al., 2005, 2012; Maberly et al., 2009) to the rule that algae and cyanobacteria possess CCMs (Beardall and Raven, 2016), so CCMs support at least 39 Pg C (80%) of annual marine net primary production (Field et al.,

1998; Raven and Beardall, 2014). CCMs also contribute at least 14 Pg C annually, mainly by $C_4$ flowering plants, to terrestrial net primary production (Field *et al.*, 1998; Still *et al.*, 2003). This means that at least 53 Pg C $year^{-1}$ of the global net primary productivity of 105 Pg C $year^{-1}$ involves CCMs rather than diffusive $CO_2$ entry (Field *et al.*, 1998; Raven *et al.*, 2012; Raven and Beardall, 2014).

In this review we discuss how CCMs respond to changes in environmental conditions and explore through this how CCMs may have evolved and persisted though major climatic shifts such as 'Snowball Earth' and how they may fare under predicted changes in our future climate.

## Response of extant cyanobacteria, eukaryotic algae, and embryophytes to environmental changes in relation to the evolution and possible future of CCMs

The objective here is to determine how changes in inorganic carbon, combined nitrogen, phosphorus, and iron concentrations, photosynthetically active radiation (PAR), and temperature alter expression of CCMs by acclimation and, in a few cases, adaptation in experimental evolution. For inorganic carbon, a major consideration for organisms with CCMs in the context of past and future high $CO_2$ episodes is the external concentration of $CO_2$ at which diffusive $CO_2$ influx gives the same photosynthetic rate as is produced by the present atmospheric $CO_2$ concentration using a CCM, assuming no change in the kinetics or content of Rubisco. The real world is more complex, and account is taken of changes in Rubisco kinetics through evolution (Young *et al.*, 2012, 2016) and of Rubisco content by changes over hours in the rates of transcription and of translation resulting in

the large observed differences in the fraction of the proteome occupied by Rubisco among extant oxygenic photosynthetic organisms (Losh *et al.* 2013; Raven 2013; Flynn and Raven 2017).

### Using the kinetics of whole-cell $CO_2$ assimilation in the absence of CCMs, and of Rubisco, in extant cyanobacteria to predict the level to which $CO_2$ would have to have fallen to make a CCM essential

Dealing first with cyanobacteria, a CCM appears to be constitutive (i.e. occurs in all photosynthetically competent species under all of the conditions so far tested), although the occurrence of the various plasmalemma and thylakoid membrane components varies phylogenetically. The minimal requirements for the cyanobacterial CCM are one energized transporter accumulating $HCO_3^-$ in the cytosol, the absence of carbonic anhydrase from the cytosol, and anion-permeable carboxysomes containing the entire intracellular carbonic anhydrase activity as well as all the Rubisco (Price and Badger 1989*a*; Hopkinson *et al.* 2014). Badger *et al.* (2002, 2006), Badger and Price (2003), and Price *et al.* (2008) pointed out that all cyanobacteria examined at the time have carboxysomes and associated mechanisms, and subsequent work has shown that 125 of the 126 cyanobacterial genomes sequenced contained coding for carboxysomal components (Abdul-Rahman *et al.* 2013; Gonzalez-Esquer *et al.* 2016). The exception is the genome of UCYN-A, a diazotrophic symbiont of marine prymnesiophycean phytoplankton that also lacks PSII and autotrophic $CO_2$ fixation by the PCRC (Zehr *et al.* 2008; Abdul-Rahman *et al.* 2013; Bombar *et al.* 2014; Cornejo-Castillo *et al.* 2016). Other cyanobacteria lacking carboxysomes are the non-photosynthetic diazotrophic endosymbionts of freshwater diatoms of the family Rhopalodiaceae; these cyanobionts

lack all photosynthetic genes (Nakayama *et al.*, 2014). All the sequenced genomes of photosynthetically competent cyanobacteria also have one or more energized transporters for $HCO_3^-$, and many have catalysed, downhill, $CO_2$ entry and unidirectional conversion of cytosolic $CO_2$ to $HCO_3^-$ energized by the NAD(P)H-PQ oxidoreductase in the thylakoid membrane; or in the plasma membrane in the thylakoid-less *Gloeobacter*. The default assumption is that all photosynthetically competent cyanobacteria grown in present atmospheric $CO_2$ express CCMs. This agrees with the low $CO_2$ affinity and $CO_2/O_2$ selectivity of the Form IB Rubisco of the ancestral (Badger *et al.*, 2002; Badger and Price, 2003) β-Cyanobacteria ($K_{0.5}$ for $CO_2$, i.e. the $CO_2$ concentration at which the enzyme achieves half of its $CO_2$-saturated rate) of 105–290 mmol m$^{-3}$; $CO_2/O_2$ selectivity 38–56; and $CO_2$-saturated specific reaction rate 2.6–11.4 site$^{-1}$ s$^{-1}$ (Jordan and Ogren, 1981; Tcherkez *et al.*, 2006; Badger and Bek, 2008; Shih *et al.*, 2016). The Form IA Rubisco of the (derived SynPro clade; Badger *et al.*, 2002; Badger and Price, 2003) α-cyanobacterium *Prochlorococcus* MIT9313 has the highest known $K_{0.5}$ for $CO_2$ of a Form I Rubisco of 750 mmol m$^{-3}$, combined with a moderate $CO_2$-saturated specific reaction rate of 4.7 site$^{-1}$ s$^{-1}$ (Scott *et al.*, 2007; see also Tcherkez *et al.*, 2006). However, Shih *et al.* (2016) found a $K_c$ of only 309 mmol m$^{-3}$ for the same strain of *Prochlorococcus* as was used by Scott *et al.* (2007), although the Rubisco was expressed in *Escherichia coli* in the work of Shih *et al.* (2016).

The low (but essential) C flux through P-glycolate and its subsequent metabolism reveals the presence of Rubisco oxygenase activity in wild-type cyanobacteria (Eisenhut *et al.*, 2008; Raven *et al.*, 2012) although the steady-state $CO_2$ concentration in the carboxysome is close to saturating for Rubisco carboxylase (assumed to be four times the

$K_{0.5}$ for Michaelis–Menten kinetics), namely 420–1160 mmol m$^{-3}$ for β-Cyanobacteria with Form IB Rubiscos and 1240–3000 mmol m$^{-3}$ for an α-cyanobacterium with Form IA Rubisco. These calculations assume that the Rubisco content is just sufficient to account for the *in vivo* inorganic C-saturated rate of photosynthesis. These values correspond to 52–116 and 124–300 times the present atmospheric concentration of $CO_2$ (dissolved in water of a temperature and salinity to give an equilibrium concentration of 10 mmol m$^{-3}$), corresponding to $K_{0.5}$ values 10.5–29 and 31–75 times the present atmospheric level, respectively. Lower atmospheric $CO_2$ values consistent with diffusive $CO_2$ entry are possible if a larger relative flux through P-glycolate is assumed, or if there are higher contents of Rubisco per cell, for example in the type II mutants or the carboxysome-less mutants of *Synechococcus* PCC7942 (Price and Badger, 1989*b*; Harano *et al*. 2003). That one or both of these effects occur is because the high $CO_2$ values used in mutant studies of the CCM in β-Cyanobacteria can be only 25-fold the present atmospheric level (Price and Badger 1989*a*, *b*), so the half-saturated value is ~25/4 or ~6 times the present atmospheric level. Badger *et al*. (2002) and Riding (2006) suggest that a value of <10 times the present atmospheric level of $CO_2$ favours cyanobacterial CCMs over diffusive $CO_2$ entry. Changed Rubisco kinetics (increased $CO_2$ affinity and $CO_2/O_2$ selectivity, preferably with an unchanged specific reaction rate, if this is possible: Tcherkez *et al*. 2006) might occur over evolutionary time of 100–1000 or more generations. While Kapralov and Filatov (2007) found no evidence for positive selection in cyanobacterial Form IB Rubiscos, their small sample size means positive selection could have been missed: as seen above, there is a significant range of kinetic properties of cyanobacterial Form IB Rubiscos.

# Using the kinetics of whole-cell $CO_2$ assimilation in the absence of CCMs, and of Rubisco, in extant eukaryotic algae to predict the level to which $CO_2$ would have to have fallen to make a CCM essential

Eukaryotic algal CCMs are very probably homoplastic, having originated independently in different clades of algae. Homoplasy can be inferred from an earlier origin of a clade than of eukaryotic CCMs, although the timing of both cladogenesis and the origin of CCMs is poorly constrained (Raven, 1997; Badger *et al.,* 2002; Raven *et al.,* 2012). Additional evidence for a homplastic origin is the diversity of inorganic C transporters and of carbonic anhydrases among eukaryotic algae, with horizontal as well as vertical gene transfer, and the lack of a strict correlation between occurrence of a CCM and the presence of a pyrenoid (Kevekordes *et al.,* 2006; Meyer and Griffiths, 2013; Raven and Giordano, 2017).

For eukaryotic algae with CCMs, the approach taken is similar to that used above for cyanobacteria. In the eukaryotic algae, there is no indicator of occurrence of a CCM like the carboxysome of cyanobacteria. For example, while the presence of pyrenoids is often associated with the occurrence of a CCM, there are a substantial number of exceptions (Badger *et al.,* 1998; Kevekordes *et al.,* 2006; Raven and Giordano, 2017). Many, or perhaps all, of the eukaryotic algal CCMs are subject to down-regulation when grown at high $CO_2$, but to avoid incomplete down-regulation of the CCM, deletion of one or more components of the CCM is needed. There are very few such measurements, and those that have been carried out are mainly on *Chlamydomonas reinhardtii*. Wang *et al.*

(2014) isolated a mutant (H82) of *C. reinhardti* that lacked expression of two CCM-related proteins (HLA3 and LCIA, operating in series in inorganic C movement from the extracellular medium to the stroma) but retained the same inorganic C-saturated photosynthetic rate as the wild type when grown at either high (50 mmol mol$^{-1}$ total gas) or low $CO_2$ (0.4 mmol mol$^{-1}$ total gas). Assuming an experimental temperature of 25 °C and allowing for the ionic strength of the experimental buffer to give a p$K_{a1}$ for inorganic C of 6.30, the $K_{0.5}$ for $CO_2$ of H82 was 14.3 mmol m$^{-3}$ and 16.6 mmol m$^{-3}$, respectively, for high and low $CO_2$-grown cells; the corresponding values for wild-type cells were 9.44 mmol m$^{-3}$ and 1.51 mmol m$^{-3}$, respectively. Assuming, as for cyanobacteria, that inorganic C-saturated photosynthesis requires four times the $K_{0.5}$ $CO_2$, this would be, respectively, 57.2 mmol m$^{-3}$ and 66.4 mmol m$^{-3}$ for high and low $CO_2$-grown cells of H82 (i.e. 5.72–6.64 times the present atmospheric $CO_2$). The external $CO_2$ concentration must be higher than the steady-state concentration of $CO_2$ at the active site of Rubisco when $CO_2$ entry is by diffusion, given the diffusive limitation to the $CO_2$ flux from the bulk medium to Rubisco imposed by membranes and path tortuosity in the aqueous phase. This has been analysed by, for example, Raven *et al*. (2005), Raven and Beardall (2016*b*), and Barbour *et al*. (2016). Supplementary Information S1 at *JXB* online, including Supplementary Table S1, provides a more detailed analysis specific to eukaryotic algal cells in the size range considered here. Such data are not available for cyanobacteria, apart from the $CO_2$ concentration needed to saturate *in vivo* photosynthesis by diffusive $CO_2$ entry, but the constraint on $CO_2$ assimilation by resistance to $CO_2$ diffusion through membranes (Raven and Beardall, 2016*b*) would be smaller in

cyanobacteria (Gram-negative outer membrane and plasmalemma) than in green algae (plasmalemma and two chloroplast envelope membranes).

Similar Rubisco kinetic data to those used in the discussion of the $CO_2$ concentration needed to saturate photosynthesis with diffusive $CO_2$ entry in cyanobacteria are also available for *C. reinhardtii*, with $K_{0.5}$ for $CO_2$ of 29–31 mmol m$^{-3}$ and $CO_2/O_2$ selectivity of 61 (Jordan and Ogren, 1981; Genkov *et al*. 2010). To achieve half of the $CO_2$-saturated rate of photosynthesis by diffusive $CO_2$ entry would thus require at least 30 mmol m$^{-3}$ $CO_2$ in the bulk phase, allowing for the necessary decrease in $CO_2$ concentration along the diffusion pathway from the bulk medium to Rubisco (i.e. equivalent to almost three times the present atmospheric concentration at sea level). Importantly, Sharkey and Berry (1985) showed that shifting *C. reinhardtii* cultures from aeration with 3300 μmol $CO_2$ mol$^{-1}$ to 200 μmol $CO_2$ mol$^{-1}$ total gas induced the CCM, meaning that the diffusive $CO_2$ entry is adequate with dissolved $CO_2$ in equilibrium with not more than 8.25 the present atmospheric level. Making the same assumption as for the cyanobacteria (i.e. $CO_2$-saturated photosynthesis needs four times the $K_{0.5}$ concentration of $CO_2$), $CO_2$ saturation of photosynthesis using a diffusive supply of $CO_2$ requires at least (allowing for the necessary decrease in $CO_2$ concentration along the diffusion pathway from the bulk medium to Rubisco, Raven *et al*. 2005) 120 mmol m$^{-3}$ dissolved $CO_2$, equivalent to almost 10 times the present atmospheric concentration at sea level. The measurements and modelling of Yokota *et al*. (1987) suggest a larger flux through Rubisco oxygenase relative to Rubisco carboxylase in *C. reinhardtii* than occurs in cyanobacteria when CCMs are functioning, and photosynthesis using diffusive $CO_2$ entry would need about twice the present atmospheric concentration of $CO_2$ to achieve half the

maximum rate of photosynthesis. This is a lower value than the factor of 4.5 based on the $CO_2$ dependence of CCM-less mutants of *C. reinhardtii* described in the previous paragraph. Kapralov and Filatov (2007) did not include the Chlorophyceae, the Class to which *C. reinhardtii* belongs, in their search for positive selection in Rubiscos; such a search, with a large sample size, would be useful.

For comparison, the freshwater Synurophyceae (and the closely related Chrysophyceae) lack CCMs (Saxby-Rouen *et al.* 1997, 1998; Bhatti and Colman 2005, 2008, 2011; Raven *et al.* 2005; Maberly *et al.* 2009). These algae have Form ID Rubiscos which are reported to have $K_{0.5}$ values (mmol m$^{-3}$) *in vitro* of 18.2 (*Mallomonas papulosa*), 28.4 (*Synura petersenii*), and 41.8 (*Synura uvella*) (Bhatti and Colman 2008). The $K_{0.5}$ values for $CO_2$ for in vivo photosynthesis for the three synurophyceans are 92.0–440.5 mmol m$^{-3}$ for *M. papillosa* (varying with the buffer used to maintain the pH at 7.0), 40.4–43.7 mmol m$^{-3}$ (varying with pH 6–7) for *S. petersenii*, and 44.9–209 mmol m$^{-3}$ for *S. uvella* (varying with pH 6–7) (Bhatti and Colman 2008). These *in vivo* values can be accommodated by diffusive $CO_2$ entry with a Rubisco content giving a $V_{max}$ for Rubisco equal to the $V_{max}$ for *in vivo* photosynthesis, granted the necessary decrease in $CO_2$ concentration along the diffusion pathway from the bulk medium to Rubisco (Raven *et al.* 2005; Raven and Beardall 2016*b*; see Supplementary Information S1) which involves passage through five membranes (plasmalemma, two chloroplast endoplasmic reticulum membranes, and two chloroplast envelope membranes) in these algae resulting from secondary plastid endosymbiosis rather than three in green algae and two in cyanobacteria (see discussion above). The Rubisco assays cited above involved unpurified cell extracts (Bhatti and Colman 2008) so the $CO_2$-saturated Rubisco-specific

reaction rate cannot be calculated. The half-saturation values for $CO_2$ fixation *in vivo* correspond to at least 2.5 times the present atmospheric concentration. Analyses by Young *et al.* (2012) show no positive selection on Rubisco from the Chrysophyceae or Synurophyceae; however, the analytical method was very conservative. There is evidence for positive selection of the Rubisco of diatoms that, like synurophyceans, are members of the Heterokontophyta (=Ochrophyta=Ochrista), but have CCMs (Young *et al.* 2012). The Rubiscos of diatoms show a range of kinetic properties, possibly related to trade-offs between Rubisco kinetics and the properties of the CCM in individual diatom species (Young *et al.* 2016).

In summary then, for cyanobacteria, the external $CO_2$ concentration needed to give half the $CO_2$-saturated rate when $CO_2$ is supplied to Rubisco by diffusion *in vivo* is estimated at 6–25 (β-Cyanobacteria) or 35–75 (α-Cyanobacteria) times the present atmospheric level. For eukaryotic algae, estimated half-saturated rate values *in vivo* are 2–2.5 times the present atmospheric level. For $CO_2$-saturated rates, the values are ~4 times higher.

## Effects of temperature on the $CO_2$ affinity (1/$K_{0.5}$) and $CO_2$/$O_2$ selectivity of Rubisco and its relationship to geographic distribution of photosynthetic organisms with and without CCMs

Here we consider terrestrial $C_4$ photosynthesis in terrestrial flowering plants as well as aquatic organisms with CCMs, since temperature as a possible factor in the evolution of CCMs was first mooted for $C_4$ plants on land. Furthermore, Raven and Beardall (2014) noted that some aquatic organisms with CCMs occur in colder habitats than do terrestrial $C_4$ plants, and their analysis is extended here.

The starting point for considering the temperature dependence of the natural occurrence of oxygenic photosynthetic organisms with CCMs and those relying on diffusive $CO_2$ entry is the temperature dependence of the kinetic properties of Rubisco and also of the solubility of $CO_2$ and $O_2$ and the kinetics, and equilibrium constants, of the dissolved $CO_2$–$HCO_3^-$–$CO_3^{2-}$–$H^+$–$OH^-$ system. Regardless of the absolute values of the $K_{0.5}$ for $CO_2$, the $CO_2/O_2$ selectivity factor, and the specific reaction rate at $CO_2$ saturation (mol $CO_2$ mol site$^{-1}$ s$^{-1}$) for a particular Rubisco, the $CO_2$ affinity ($1/K_{0.5}$ $CO_2$) and $CO_2/O_2$ selectivity factor both increase with decreasing temperature, while the specific reaction rate decreases (Ehleringer et al., 1991; Tcherkez et al., 2006; Edwards and Still, 2008; Galmés et al., 2016). The temperature effect on $K_{0.5}$ for $CO_2$ and $CO_2/O_2$ selectivity, and also the greater rate of increase of $CO_2$ solubility than of $O_2$ solubility with decreasing temperature (Ku and Edwards 1977), has been related to the occurrence of $C_4$ terrestrial flowering plants in higher temperature environments where higher $K_{0.5}$ for $CO_2$, lower $CO_2/O_2$ selectivity factor, and the lower $CO_2:O_2$ solubility ratio at higher temperatures increases the relative rate of Rubisco oxygenase and hence photorespiration in $C_3$ plants, and hence favours the occurrence of $C_4$ (a biochemical form of CCM) (Ehleringer et al., 1991; Edwards and Still 2008). For low temperatures, the prediction is a lower selective value of CCMs (Ehlringer et al., 1991; Raven et al., 2002a, b; Edwards and Still, 2008), though the evidence discussed below suggests that CCMs of cyanobacteria and algae can occur in cold habitats. We note that there is some evidence of down-regulation of CCMs in Antarctic marine diatoms (Kranz et al., 2015), and also that there is no evidence of adaptive changes to the Rubisco-specific reaction rate in Antarctic diatoms (Young et al., 2015b) despite the known variability of Rubisco kinetics

among diatoms (Young *et al*. 2016) and the sea close to the Antarctic having had its present low temperatures for at least 15 million years (Raven *et al*. 2002*b*).

Expanding on Raven and Beardall (2014), Table 1 shows that terrestrial C$_4$ (and CAM plants, also using a biochemical CCM) do not extend to such high latitudes and altitudes (i.e. lower temperatures, among other differences) as do C$_3$ flowering plants and other C$_3$ terrestrial embryophytes. Table 1 also shows that marine, freshwater, and terrestrial cyanobacteria and algae (free-living and lichenized) with CCMs are much more significantly represented in high latitudes and (for freshwater and terrestrial organisms) high altitudes than is the case for terrestrial C$_4$ and CAM plants. Certainly some Antarctic microalgae (Mitchell and Beardall, 1996) and macroalgae (Beardall and Roberts, 1999) express CCMs despite the low temperatures and higher dissolved CO$_2$ levels in their high latitude environments; see also Johnston and Kennedy (1998), Raven *et al*. (2002*a, b*), Marconi *et al*. (2011), Kranz *et al*. (2015), Stepien (2015), Young *et al*. (2015a, *b*) and Stepien *et al*. (2016), noting that the $^{13}$C:$^{12}$C cut-off for assignment to 'CO$_2$ diffusion only' may need revision for some algae with Form ID Rubiscos (Boller *et al*. (2011, 2015). This current distribution is relevant to consideration of what might have happened in the past and might happen in the future, granted the mechanistic positive relationship (other things being equal) between increased atmospheric CO$_2$ and increased temperature.

# Effects of incident PAR and the concentration of combined nitrogen, phosphorus, and iron on the expression of CCMs in organisms in which the CCM expression is not constitutive

We regard CCMs as constitutive if they are always expressed under the conditions that the organisms encounter in their natural environment, as well as at larger $CO_2$ concentrations than are found in those environments. By this definition, all photosynthetically competent cyanobacteria, some terrestrial CAM plants, and almost all terrestrial $C_4$ plants have a constitutive CCM. The exception for the $C_4$ plants is the sedge *Eleocharis*, where submergence causes some $C_4$ species to switch to $C_3$ or $C_3$–$C_4$ intermediate metabolism (Ueno *et al*. 1988; Murphy *et al*. 2007). Ontogenetic changes from $C_3$ cotyledons to $C_4$ in the leaves and/or stems in some members of the Chenopodiaceae are not environmentally determined (Pyankov *et al*. 1990, 2000).

Organisms in which the extent of expression of CCMs varies with the supply of resources are algae with biophysical CCMs, some CAM plants, and some submerged and amphibious tracheophytes with CCMs. Here we deal with algae, based on the literature analysis in Raven and Beardall (2014) and the earlier reviews of Beardall and Giordano (2002) and Raven *et al*. (2000).

Decreased PAR decreases the $CO_2$ accumulation in cells of the cyanobacterium *Anabaena variabilis* (with a constitutive CCM) (Beardall 1991; Raven and Beardall 2014). In this cyanobacterium and in two marine red macroalgae and the green marine (to hypersaline) alga *Dunaliella tertiolecta*, there is a smaller increment of photosynthetic rate per unit increment in inorganic C concentration (i.e. a lower affinity for inorganic C) at low PAR relative to high PAR for growth, although the reverse is true for a seagrass that may have a CCM (Kübler and Raven 1995; Young and Beardall 2005; Raven and Beardall 2014). In most cases, the natural abundance $^{13}C:^{12}C$ ratio of organic C of algae with CCMs is higher at low PAR, consistent with, among other possibilities, increased

$CO_2$ leakage as a fraction of inorganic C entering at low PAR, thus decreasing the energetic effectiveness of the CCM (Raven *et al.* 2000; Beardall and Giordano, 2002; Raven and Beardall, 2014, 2016*b*). Overall, the data support a less effective CCM at low PAR for the cyanobacterium and the algae examined.

Stepien (2015) performed a meta-analysis of data on the natural abundance $^{13}C$:$^{12}C$ ratio of organic C of marine macroalgae collected from their natural habitats and showed that at greater depths (low PAR) a greater proportion of algae showed very low $^{13}C$:$^{12}C$ ratios, indicative of diffusive $CO_2$ transport from bulk seawater to Rubisco. It is not clear whether the very deep-growing marine macroalgae have CCMs; the deepest growing red coralline alga at 268 m is only exposed to PAR of ~2 nmol photon $m^{-2} s^{-1}$ (Raven *et al.* 2000; Runcie *et al.* 2008), with full sunlight at the ocean surface of up to $10^6$-fold higher at 2 mmol $m^{-2} s^{-1}$ PAR. It is very unlikely that the algae are supplementing photosynthesis with phagotrophy of organic C, though use of dissolved organic C has not been ruled out (Raven *et al.* 2000). For cyanobacteria, with their constitutive CCMs, the planktonic *Prochlorococcus* is a, and usually the, predominant photosynthetic organism in the Deep Chlorophyll Maximum peaks in the tropical and subtropical ocean at 80–100 m with incident PAR of ~10 µmol photons $m^{-2} s^{-1}$, although outliers at greater depths may decrease that value to not less than 1 µmol photons $m^{-2} s^{-1}$ (Campbell and Vaulot, 1988; Smith *et al.* 1989; Letelier *et al.* 2004; Casey *et al.* 2007). Cyanobacteria may grow at lower PAR in cryptoendolithic environments with a PAR range imposed by self-shading of 150–0.1 µmol photons $m^{-2} s^{-1}$ since the stratification of the organisms is not known (Nienow *et al.* 1988).

Data on nutrient supply effects on CCMs from Raven and Beardall (2014) are summarized in Table 2. For combined N limitation, there is variability in the data for the influence of N concentration both on the CCM and on the N use efficiency of growth, the latter generally being higher in $C_4$ than in $C_3$ flowering plants. P limitation usually (three out of five instances, including the most detailed data set) decreases CCM expression. Finally, the only data set for the effects of Fe deficiency shows an increased CCM expression under Fe limitation (Table 2; Young and Beardall, 2005). Overall, the data show a decreased CCM expression when PAR or P are limiting, an increased CCM expression when Fe is limiting, and variable effects of combined N deficiency or decreased UV. These short-term, acclimatory, effects have relevance to altered surface ocean stratification with global warming, as is occurring at the moment, but not necessarily to long-term evolutionary effects in the more distant future or in the past.

## Evolution of CCMs and the possibility of their continuation through both 'Snowball Earth' and high $CO_2$ 'hothouse' episodes in Earth history

The focus here is on oxygenic photolithotrophic organisms, although a core part of the cyanobacterial CCM, the carboxysome, also occurs in autotrophic proteobacteria (anoxygenic photosynthesizers and chemolithotrophs) that use Rubisco and the PCRC (Raven *et al.*, 2012). Mechanisms in oxygenic photosynthetic organisms that metabolize the unique product, phosphoglycolate, formed from the oxygenase activity of Rubisco, that go beyond the excretable (in aquatic organisms) immediate product of phosphoglycolate metabolism, glycolate, are ubiquitous in oxygenic organisms and are

essential even in organisms using CCMs (Raven *et al.* 2012). This is consistent with a pre-CCM occurrence of phosphoglycolate production that in turn requires the occurrence of oxygen in the organism's environment. The predominant source of $O_2$ on Earth is oxygenic photosynthesis, and the earliest oxygenic organisms were what in extant organisms are cyanobacteria, so the temporal sequence was (Raven *et al.* 2012):

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oxygenic photosynthesis in cyanobacteria or their ancestors

↓

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production of phosphoglycolate and its metabolism beyond glycolate

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↓

CCMs

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While the timing of origin for the crown group of extant cyanobacteria is still debated (Shih *et al.* 2016), evolutionary studies agree that oxygenic photosynthesis must have evolved before the Global Oxygenation Event (GOE) some 2.3 Ga (Blank and Sánchez-Baracaldo 2010; Sánchez-Baracaldo 2015; Schirrmeister *et al.* 2016): Figs 1–3. Other lines of evidence are consistent with oxygenic photosynthetic organisms appearing at least 2.7 Ga (e.g. Buick 1992, 2008; Schirrmeister *et al.* 2015, 2016). It is unclear whether Proterozoic cyanobacteria resemble extant taxa; however, the oldest reliable cyanobacteria microfossils appeared in carbonate strata ~1.9 Ga in the Belcher Islands, Canada (Hofmann 1976). Based on phylogenetic analyses, we now know that the sister clade of the cyanobacteria is the non-photosynthetic Melainabacteria, many of which are purely fermentative, while some have anaerobic and, possibly, aerobic respiration (Di Rienzi *et al.* 2013; Soo *et al.* 2014). There are views explaining how PSII and PSI of oxygenic photolithotrophy reached the ancestral cyanobacterium. One hypothesis

involves lateral gene transfer of, respectively, a Type II reaction centre from a proteobacterium, and a Type I reaction centre from a member of the Chloroflexi, together with their associated peptides, as well as those of the (bacterio)chlorophyll synthesis pathway and of the PCRC (Soo *et al.* 2014). A second hypothesis proposes that both PSII and PSI evolved in an ancestral phototroph as a result of gene duplication. Consequently anoxygenic phototrophs selectively lost one of the photosystems, while cyanobacteria retained both (Cardona 2015). Here, it is implied that phototrophy is mostly vertically inherited and that non-phototrophs (e.g. Melainabacteria, Soo *et al.* 2014) that evolved from a phototroph lost both photosystems.

The basal extant cyanobacterium is the β-cyanobacterium (Badger *et al.* 2002; Badger and Price 2003) *Gloeobacter*, possessing Form IB Rubisco, β-carboxysomes and associated inorganic C transporters, which grows in low-salinity water films on limestone and dolomite (*G. violacea*, Nakamura *et al.* 2003; Horath and Bachofen 2009; Mareš *et al.* 2013) or igneous rocks (*G. kiluaeensis*, Saw *et al.* 2013). Independent phylogenomic studies, including both protein and nucleotide data and implementing a relaxed Bayesian molecular clock approach, suggest that *Gloeobacter* could have evolved before the GOE (Blank and Sánchez-Baracaldo 2010; Sánchez-Baracaldo *et al.* 2014; Sánchez-Baracaldo 2015; Schirrmeister *et al.* 2015 2016; see also Dillon and Castenholz 1999; Olsson-Francis *et al.* 2010). An opposite view from Butterfield (2015; see also Lyons *et al.* 2014) is that extant cyanobacteria evolved later as crown group Cyanobacteria with no basal or stem group Cyanobacteria extant. According to this view, the environment in the late Archaean and Proterozoic may be irrelevant to the origin and perpetuation of the cyanobacterial CCM.

Assuming that phylogenomics and Bayesian molecular clock analyses are correct, and that *Gloeobacter* has retained features present in the basal cyanobacterium, it is possible that cyanobacteria at the time of the GOE had CCMs. This seems contrary to the need for greenhouse gases at greater than present, and recent (10 Ma) partial pressures if a Snowball Earth is to be avoided given the lower than present luminosity of the Faint Young Sun (Claire *et al.* 2012; see also Som *et al.* 2016). One such gas is $CO_2$, and another, $CH_4$, was probably at low partial pressures after the GOE even with low atmospheric $O_2$ as an oxidant for $CH_4$ (Olson *et al.* 2016). This would argue for relatively high $CO_2$ concentrations through most of the Proterozoic, with the exception of the Palaeoproterozoic (Huronian) and Neoproterozoic (Sturtian, Marinoan, and Gaskiers) glaciations of the Snowball/Slushball Earth episodes (Hoffman 2016); Figs 1, 2. Riding (2006) suggests that $CO_2$ was 25 times the present atmospheric level at 1.4–1.3 Ga, based on the geochemistry of $CaCO_3$ precipitation; Kah and Riding (2007) suggest that $CO_2$ could be as low as 6–10 times the present atmospheric level, assuming 100–200 ppm $CH_4$ in the atmosphere (but see Olson *et al.* 2016 who suggest $CH_4$ <10 ppm) (Fig. 2). A further consideration for the evolution of marine CCMs is the variation in ocean pH over the last 4 Ga (Halevy and Bachan 2017).

Accepting the timings of cyanobacterial evolution in Sánchez-Baracaldo *et al.* (2014; see also Lyons *et al.* 2014; Butterfield 2015), *Gloeobacter*-like organisms could have had CCMs during all of the Neoproterozoic glacial periods and this could have included, for the three Neoproterozoic glaciations, open ocean planktonic cyanobacteria. Eukaryotic photosynthetic organisms are first known from marine deposits from ~1.6 Ga (Bengtson et al. 2017) and the bangiophycean red alga *Bangiomorpha pubescens* from

~1.2 Ga (Butterfield, 2000), and freshwater and terrestrial eukaryotes, some of them probably photosynthetic, from 1.2 Ga to 1.0 Ga (Strother *et al.*, 2011; Wacey *et al.*, 2016). Oehler (1977) interprets structures in 740–950 Ma cells as pyrenoids, although there are alternative explanations of the nature of the structures, and the correlation of pyrenoids with CCMs is not exact (Kevekordes *et al.*, 2006; Meyer and Griffiths, 2013; Raven and Giordano, 2017). If at least some of the Proterozoic glaciations were at the more extreme end of the glaciological possibilities for global ice cover, it is very difficult to see how marine photosynthetic organisms could have survived. For cyanobacteria, the lower limit for growth may be not less than 1 $\mu$mol photon m$^{-2}$ s$^{-1}$ of PAR as discussed above (Campbell and Vaulot, 1988; Smith *et al.*, 1989; Letelier *et al.*, 2004; Casey *et al.*, 2007). This would be achieved under a few metres of ice containing dust and gas bubbles rather than a kilometre of ice. In contrast, freshwater and, perhaps, terrestrial organisms could have survived on the glacial surface in freshwater-filled 'cryoconite holes' produced by dust (cryoconite) from vulcanism and (to a small extent) meteorites, decreasing the albedo and allowing warming (Vincent *et al.*, 2000; Hoffman, 2016). Phylogenomic and trait evolution analyses have recovered clades common to Arctic, Antarctic, and alpine regions; these clades of cyanobacteria from terrestrial/freshwater clades probably evolved the ability to cope with such extreme cold environments independently (Chrismas *et al.*, 2015, 2016). Bayesian statistical analyses have identified at least 20 clades with high probability of having a cold-adapted ancestor (Chrismas *et al.*, 2015). While molecular clock analyses are yet to provide any dates of when these groups evolved, it is likely that some of these 'cold-adapted' clades date as far back to the Neoproterozoic glaciations. Furthermore, geological evidence and models shows habitat

availability for atmosphere-exposed marine and terrestrial habitats (Campbell *et al.* 2014; Fairchild *et al.* 2015; Retallack *et al.* 2015) and there is biomarker evidence of green sulphur bacteria, cyanobacteria, and probable eukaryotes in organic-rich shales (Olcott *et al.* 2005) as well as palaeontological evidence of marine macroalgae (Ye *et al.* 2015) occurring in the glacial episodes in the Cryogenian. These lines of evidence showing the occurrence of cyanobacteria and of eukaryotic algae as photosynthetic primary producers in the glacial episodes of the Cryogenian are consistent with the geologically (not depending on biological evidence) recognized possibility of a Slushball Earth, with ice-free low latitude marine and coastal habitats, rather than more extreme Snowball Earth conditions (Hoffman and Schrag 2002).

The previous section suggests that a CCM is needed to give half the rate of $CO_2$-saturated photosynthesis in a cyanobacterium with the highest extant cyanobacterial Form IB Rubisco $CO_2$ affinity when the atmospheric $CO_2$ is $<12$ times the present atmospheric level. If the requirement is for the rate of $CO_2$-saturated photosynthesis, the necessary $CO_2$ concentration is 48 times the present atmospheric $CO_2$ level. For eukaryotes with Form IB Rubisco, only involved in the Cryogenian, the equivalent values are as low as two and eight times the present atmospheric level. The presumed low atmospheric $CO_2$ during the glacial episodes has not been adequately modelled. The atmospheric greenhouse gases would have been higher than would be needed today for the same extent of glaciation as a result of the lower luminosity of the weak young sun, especially in the Paleoproterozoic Huronian (Claire *et al.* 2012). While extreme glaciations in the Proterozoic might give $CO_2$ concentrations consistent with the evolution of CCMs, it is less clear what would happen during the warmer time between the Huronian and

Cryogenian, especially in view of the low modelled levels of the more powerful greenhouse gas, $CH_4$ (Olson *et al.* 2016). As noted above, Riding (2006) suggests 25 times the present atmospheric level for $CO_2$ at 1.4–1.3 Ga, with the Kah and Riding (2007) value of 6–10 times the present level assuming 100–200 ppm $CH_4$ in the atmosphere rendered unlikely by the arguments of Olson *et al.* (2016) for $CH_4 <10$ ppm when $O_2$ (oxidant of $CH_4$) was relatively low (Figs 2, 3).

There are more proxies for $CO_2$ for the Phanerozoic, and especially after tracheophytes became significant components of the terrestrial flora, and even earlier with non-tracheophyte flora (Berner 2004; Breecker *et al.* 2010; Lenton *et al.* 2012; Franks *et al.* 2014; Royer 2014; Wellman and Strother 2015; McElwain *et al.* 2016; Lenton and Daines 2017). Breecker *et al.* (2010) suggest that the mean of estimates of the $CO_2$ concentration in the warmest episode of the Mesozoic were only 2.5 times the present atmospheric value, and McElwain *et al.* (2016) consider a similar value (1000 µmol $CO_2$ $mol^{-1}$ total gas, as compared with 400 µmol $CO_2$ $mol^{-1}$ total gas today) as a possible cap on $CO_2$ over the last 300 million years (i.e. since the late Palaeozoic glaciation; Figs 1–3). This would permit not only cyanobacterial CCMs, but possibly also eukaryotic CCMs, to survive had the cyanobacterial and eukaryotic CCMs evolved in the low $CO_2$ episode ($CO_2$ at about the present level) of the Carboniferous–Permian (fig. 5.21 of Berner 2004; Breecker *et al.* 2012), or earlier (e.g. the Cryogenian). However, there is very considerable variance in the estimates. (Berner 2004; Breecker *et al.* 2010), and Berner (2004) estimates five times the present $CO_2$ level in the Mesozoic. Lenton *et al.* (2012) modelled atmospheric $CO_2$ at the time of the Ordovician glaciations as 6–8 times

the present value, and before the glaciations at 16 times the present, similar to values in fig. 5.21 of Berner (2004) (Figs 2, 3).

A possible refuge for organisms with CCMs during episodes of globally high $CO_2$ is produced by the organisms themselves. The relatively slow exchange of $CO_2$ between water bodies and the atmosphere, and between parcels of water within the water bodies, means that $CO_2$ can be drawn down to values less than a third of the air-equilibrium values at times of high ecosystem primary productivity. Freshwater phytoplankton (Maberly, 1996) can decrease $CO_2$ to air-equilibrium equivalent values of <20 μmol $mol^{-1}$ and marine phytoplankton (Codispoti *et al*. 1982, 1986) to 125 μmol $mol^{-1}$, and marine macrophyte beds (Delille *et al*. 2000) can decrease $CO_2$ to air-equilibrium equivalent values of 20 μmol $mol^{-1}$. The presence of a CCM could be of importance at these times of high productivity even with high global $CO_2$ concentrations; the largest drawdowns observed today would yield the present atmospheric equilibrium values with five times the present $CO_2$ content, although this would be partly offset by the higher total inorganic C concentration under higher atmospheric $CO_2$ so that more inorganic C would have to be assimilated to get down to the present air-equilibrium $CO_2$. This effect is much less marked, with a maximum drawdown to 86% of the tropospheric $CO_2$ within a *Zea mays* canopy (Buchman and Ehleringer, 1998) as a result of greater mixing with the bulk atmosphere than in aquatic habitats.

Comparison of the estimates of $CO_2$ over the last 2 Ga with the estimates of the $CO_2$ required for diffusive $CO_2$ entry to support the $CO_2$-saturated growth rate, and the $K_{0.5}$($CO_2$) for growth, of cyanobacteria and chlorophytes that currently operate CCMs allows the following conclusions about retention of CCMs. The ancestral β-

Cyanobacteria (Form IB Rubisco and β-carboxysomes; Badger and Price, 2003) might, and the derived α-Cyanobacteria (Form IA Rubisco and α-carboxysomes: the SynPro clade; Badger and Price, 2003) could have retained CCMs throughout the Phanerozoic, and possibly from the late Mesoproterozoic, had the CCMs evolved before the Cryogenian. For the chlorophytes, had they evolved that early, the possibilities of retention of CCMs through the mid-Palaeoproterozoic are minimal, although the post-Carboniferous may not pose an insuperable problem for CCM retention.

An origin of the biophysical CCMs later than diversification of the clades in which they occur would involve further horizontal gene transfer. For cyanobacteria, an origin in the Cryogenian or later would, on any of the suggested timings of cyanobacterial diversification, involve significant horizontal gene transfer in addition to that replacing β-carboxysomes with Form IB Rubisco with α-carboxysomes with Form IA Rubisco (Badger $et\ al.$, 2002, 2006; Badger and Price, 2003; Price $et\ al.$, 2008; Sánchez-Baracaldo $et\ al.$, 2014; Butterfield, 2015). The biophysical CCMs of eukaryotic algae are more diverse than those of cyanobacteria (Badger $et\ al.$, 1998; Meyer and Griffiths, 2013; Clement $et\ al.$, 2016), but there is probably significant horizontal gene transfer involved in an origin as late as the Carboniferous low $CO_2$ and high $O_2$ period (Raven, 1997; Badger $et\ al.$, 1998, 2002; Badger and Price, 2003; see Fig. 1a and b), granted the timing of diversification of algae (Butterfield, 2010; Baurain $et\ al.$, 2010; Brown and Sorhanus, 2010; Strother $et\ al.$, 2011; Yang $et\ al.$, 2012). An even later origin (Palaeogene) is possible for the biophysical CCM of the hornworts (Villareal and Renner, 2012). $C_4$ flowering plants also originated in the Palaeogene; this biochemical CCM may have involved limited horizontal gene transfer to account for the ≥60 independent origins

of flowering plant $C_4$, at least as far as the enzymes involved in variants on the $C_3$–$C_4$ cycle are concerned (Still *et al.* 2003; Aubry *et al.* 2011; Chi *et al.* 2014). Limited horizontal gene transfer would also be the case for the possible occurrence of $C_4$ photosynthesis in algae (Koch *et al.* 2013; Clement *et al.* 2016; Raven and Giordano 2017).

## Possible future of CCMs

For marine organisms, the immediate (until 2100) effects of global environmental change in the surface ocean influence the availability of many resources. The increased atmospheric $CO_2$ means an increase in the concentration of $CO_2$, a smaller relative increase in the concentration of $HCO_3^-$ and a decrease in the concentration of $CO_3^{2-}$ and in the pH. The increased ocean temperature results initially in an increased surface (upper mixed layer) ocean temperature relative to the deeper ocean, with a larger temperature difference across, and a shoaling of, the thermocline, with a decreased flux of $NO_3^-$, $HPO_4^{2-}$ and other nutrients from the deeper ocean to the upper mixed layer (Doney *et al.* 2012; Gao *et al.* 2012; Reusch and Boyd 2013; Raven and Beardall 2014). In addition, the decreased thickness of the upper mixed layer means that phytoplankton are exposed to a higher mean flux of PAR and of UV radiation (Doney *et al.* 2012; Gao *et al.* 2012; Reusch and Boyd 2013; Raven and Beardall 2014), with higher UVB and lower nutrient levels potentially causing a shift in the size structure of phytoplankton populations favouring smaller organisms such as the cyanobacteria *Prochlorococcus* and *Synechococcus* (Finkel *et al.* 2010). In the longer term (1000–100 000 years), usable fossil fuel reserves will all have been consumed, and shoaling of the lysoclines for calcite and aragonite will weaken as $CO_2$-enriched water circulates deep into the ocean and

dissolves sedimented $CaCO_3$, thereby increasing the carbonate alkalinity of the seawater that, when it is upwelled to the surface, will absorb some or much of the anthropogenic atmospheric $CO_2$ released from fossil fuel burning and land use change (Archer *et al.*, 2009). Furthermore, the downwelled heat from the surface ocean will eventually increase the temperature of the deep ocean and decrease the temperature difference across the thermocline, allowing an increase in the thickness of the upper mixed layer. This combination of changes will thus, in time, produce an oceanic state similar to the pre-industrial ocean.

The multiplicity of changing and interacting environmental influences makes it difficult to carry out short-term studies of the acclimation of phytoplankton to these factors: what has been accomplished is summarized by Gao *et al.* (2012), Beardall *et al.* (2014), and Raven and Beardall (2014). A subsequent experiment with eight drivers, including increased $CO_2$, on *C. reinhardtii* had the final sample at 120 h (Brennan and Collins, 2015).

The complications of multifactorial experimentation are even more of a problem with longer term experimental evolution studies of genetic change. It is therefore not surprising that most work on experimental evolution has involved only a single variable of increased $CO_2$. As a simplifying factor, cultures were initiated with clonal algal inocula; even so, epigenetic changes as well as genetic changes can occur, potentially complicating interpretation (Kronholm and Collins, 2016). Following the pioneering work of Collins and Bell (2004), what has been achieved subsequently is summarized by Low-Décarie *et al.* (2013), Reusch and Boyd (2013), Schlüter *et al.* (2014), Li *et al.* (2016), and Schlüter *et al.* (2016). The longest experiment (4 years, 2100 generations;

Schlüter *et al.* 2016) examined the response of an 'adapted' population of *Emiliania huxleyi* by testing how they responded to being returned to the original $CO_2$ conditions and hence the occurrence of genetic changes, either by strain selection or mutation, or, for clonal cultures, genetic mutation alone. In the work reported by Schlüter *et al.* (2016), some traits that showed selective changes under high $CO_2$ reverted when cells were placed back in present day $CO_2$ levels, but others, such as the PIC/POC ratio, overcompensated under such conditions, showing that adaptive (genetic) changes had occurred. Particularly interesting is the work on $N_2$ fixation by the marine cyanobacterium *Trichodesmium* exposed to elevated $CO_2$ for 4.5 years (~850 generations) (Hutchins *et al.* 2015). Consistent with the results of short-term (2 week) incubations, the 4.5 year exposure cultures showed significantly higher $N_2$ fixation rates and specific growth rates under P-limited conditions, as well as shifts in the diel occurrence of peak $N_2$ fixation; this occurred for all six biological replicate cultures. These three effects continued when the cultures were returned to present $CO_2$ levels for 2 years. Analysis of the proteome and enzyme activities did not, however, reveal the basis of these changes (Hutchins *et al.* 2015; Wallworth *et al.* 2016).

Schaum and Collins (2014) examined 16 strains of *Ostreococcus* and showed that strains with a greater acclimatory response to increased $CO_2$ also showed a greater adaptive (genetic change) response to increased $CO_2$. Padfield *et al.* (2016) investigated the changes to metabolism of the freshwater *Chlorella vulgaris* over 100 generations of exposure to a higher temperature, but without demonstrating that the effects are due to adaptation rather than acclimation. Schlüter *et al.* (2014) examined increased temperature and increased $CO_2$, separately and together, on *E. huxleyi* and showed that there was no

interaction of $CO_2$ and temperature. Bermúdez *et al*. (2015) used a fully factorial design (three $CO_2$ concentrations, two temperatures) to study the amino and fatty acid composition of *Cylindrotheca fusiformis* over 250 generations and showed significant changes in polyunsaturated fatty acid content with both $CO_2$ and temperature, although again the authors were not able to distinguish between acclimatory and adaptive changes.

Whereas transcriptomic data are available for some of the acclimation and adaptation studies (e.g. Lohbeck *et al*. 2014), there are no genomic data for the adaptation experiments and the mutations involved are not documented at the molecular genetic level. In addition to the need for these data, additional environmental factors should be investigated by experimental evolution experiments. Interesting possibilities for predicting the adaptation of phytoplankton strains comes from the work of Schaum and Collins (2014) and Schaum *et al*. (2016). Schaum and Collins (2014) examined 16 strains of *Ostreococcus* and showed that strains with a greater acclimatory response to increased $CO_2$ also showed a greater adaptive (genetic change) response to increased $CO_2$. Schaum *et al*. (2016) used the same strains of *Ostreococcus* to show that exposure to temporally varying increased $CO_2$ showed a more rapid response to further increases in $CO_2$ than for treatments with constant high $CO_2$ (see also Doblin and van Sebille, 2016). Determination of whether these responses occur for other microalgae, and for other aspects of global environmental change, is required. The interactive effects of the various aspects of global change also need to be taken into account (see, for example, Boyd *et al*. 2016*a*, *b*). Finally, all of these sources of laboratory data must eventually be related to natural ecosystems (Brodie *et al*. 2014; Mock *et al*. 2016).

A beginning has been made by Scheinin *et al.* (2015), who deployed the centric diatom *Skeletonema costatum* in control and $CO_2$-enriched mesocosms in the natural environment of a fjord on the west coast of Sweden for 107 d; there was a 1.3-fold increased growth in high $CO_2$-evolved mesocosms. This contrasts with the findings for the pennate diatom *Phaeodactylum tricornutum* grown for ~1860 generations (Li *et al.* 2016) where the cultures supplied with increased $CO_2$ had lower growth, photosynthesis, and respiration rates than did the controls.

Perhaps the ultimate in long-term high $CO_2$ treatments for comparison with growth in present day $CO_2$ is freshwater springs (Collins and Bell, 2006) and marine shallow water vents emitting mainly, or solely, $CO_2$ as the gas dissolved in the emerging water (Porzio *et al.* 2011, 2013; Johnson *et al.* 2012). There are a number of issues with such systems in comparison with laboratory work. One is the problem of finding comparative ecosystems for the planktonic and benthic algal communities of freshwater springs. This problem seems smaller for benthic macroalgae close to marine vents where the surrounding habitats differ (mainly) in $CO_2$ input, but there can be exchange of genotypes between the vents and the surroundings, as well as differential effects on epiphyte settlement and on motile macroalgal genotypes (Porzio *et al.* 2011, 2013; Johnson *et al.* 2012).

## Conclusions

CCMs, based on active transport of inorganic carbon or, in vascular plants, on $C_4$ and CAM biochemistry, support over half of the planet's primary productivity. In algae and cyanobacteria there is considerable diversity in the kinetic properties of Rubisco, but data based on the $CO_2$ level needed to support half-saturated rates of C fixation suggest that

evolution of CCMs would have been favoured when atmospheric $CO_2$ levels were <16 times (cyanobacteria) or as low as <2 times present day values. CCM activity in extant photoautotrophs is regulated by a range of environmental factors, including temperature, light, and nutrient availability. This information allows us to make some predictions about CCM activity and evolutionary changes in a future climate, though the complexity of interactions between environmental factors will necessitate more complex experimental approaches than have been employed in the past if we are to obtain an accurate picture of how CCMs and primary productivity will respond in the next century and beyond.

## Supplementary data

Supplementary data are available at *JXB* online.

**Table S1.** Comparison of $K_{0.5}$ values (mmol m$^{-3}$) for $CO_2$ assimilation by whole cells with $K_{0.5}$ values (mmol m$^{-3}$) for $CO_2$ transformation into 3-phosphoglycerate by extracted Rubisco for three species of the Synurophyceae and one of Trebouxiophyceae lacking CCMs.

Information S1. Diffusion limitations on photosynthesis by algae lacking CCMs

## Acknowledgements

## References

**Abdul-Raman F, Petit E, Clanchard JL,** 2013. The distribution of polyhedral bacterial microcompartments suggests frequent horizontal transfer and reassembly. Journal of Phylogenetics and Evolutionary Biology **1**, 4.

**Archer D, Eby M, Brovkon V,** *et al.* 2009. Atmospheric lifetime of fossil fuel carbon dioxide. Annual Review of Earth and Planetary Sciences **37**, 117–134.

**Aubry S, Brown NJ, Hibberd JM.** 2011. The role of proteins in $C_4$ plants prior to their recruitment into the $C_4$ pathway. Journal of Experimental Botany **62**, 3049–3059.

**Badger MR, Andrews TJ, Whitney SM,** *et al.* 1998. The diversity and co-evolution of Rubisco, plastids, pyrenoids and chloroplast-based $CO_2$ concentrating mechanisms in algae. Canadian Journal of Botany **76,** 1052–1071.

**Badger MR, Bek EJ.** 2008. Multiple Rubisco forms in proteobacteria: their functional significance in relation to $CO_2$ acquisition by the CBB cycle. Journal of Experimental Botany **59**, 1525–1541.

**Badger MR, Hanson DT, Price GD.** 2002. Evolution and diversity of $CO_2$ concentrating mechanisms in cyanobacteria. Functional Plant Biology **29**, 407–416.

**Badger MR, Price GD.** 2003. $CO_2$ concentrating mechanisms in cyanobacteria: molecular components, their diversity and evolution. Journal of Experimental Botany **54**, 609–622.

**Badger MR, Price GD, Long BM, Woodger FJ.** 2006. The environmental plasticity and ecological genomics of the cyanobacterial $CO_2$ concentrating mechanism. Journal of Experimental Botany **57**, 249–265.

**Barbour MM, Evans JR, Simonin KA, von Caemmerer S.** 2016. Online $CO_2$ and $H_2O$ oxygen isotope fractionation allows estimation of mesophyll conductance in C4 plants, and reveals that mesophyll conductance decreases as leaves age in both C4 and C3 plants. New Phytologist **210**, 875–889.

**Baurain D, Brinkmann H, Petersen J, Rodríguez-Ezpeleta N, Stechmann A, Demoulin V, Roger AJ, Burger G, Lang BF, Philippe H.** 2010. Phylogenomic evidence for separate acquisition of plastids in cryptophytes, haptophytes, and stramenopiles. Molecular Biology and Evolution **27**, 1698–1709.

**Beardall J.** 1991. Effects of photon flux density on the '$CO_2$ concentrating mechanism' of the cyanobacterium *Anabaena variabilis*. Journal of Plankton Research **13**, 133–142.

**Beardall J, Giordano M.** 2002. Ecological implications of microalgal and cyanobacterial $CO_2$ concentrating mechanisms and their regulation. Functional Plant Biology **20**, 335–340.

**Beardall J, Griffiths H, Raven JA.** 1982. Carbon isotope discrimination and the $CO_2$ accumulating mechanism in *Chlorella pyrenoidosa*. Journal of Experimental Botany **33**, 729–737.

**Beardall J, Raven JA.** 2016. Carbon acquisition by algae. In: Borowitzka M, Beardall J, Raven JA, eds. The physiology of microalgae. Heidelberg: Springer, 89–99.

**Beardall J, Roberts S.** 1999. Inorganic carbon acquisition by two species of Antarctic macroalgae: *Porphyra endivifolium* (Rhodophyta: Bangiales) and *Palmaria decipiens* (Rhodophyta: Palmariales). Polar Biology **21**, 310–315.

Beardall J, Roberts S, Millhouse J. 1991. Effects of nitrogen limitation on uptake of inorganic carbon and specific activity of ribulose-1,5-bisphosphate carboxylase-oxygenase in green microalgae. Canadian Journal of Botany **69**, 1146–1150.

Beardall J, Roberts S, Raven JA. 2005. Regulation of inorganic carbon acquisition by phosphorus limitation in the green alga *Chlorella emersonii.* Canadian Journal of Botany **83**, 859–864.

Beardall J, Stojkovic S, Gao K. 2014. Interactive effects of nutrient supply and other environmental factors on the sensitivity of marine primary producers to ultraviolet radiation: implications for the impacts of global change. Aquatic Biology **22**, 5–23.

Bengtson S, Salistedt T, Belivanova V, Whitehouse M. 2017. Three-dimensional preservation of cellular and subcellular structures suggests 1.6 billion-year-old crown-group red algae. PLOS Biology **15**, e2000735.

Bermúdez R, Feng Y, Roleda MY, *et al*. 2015. Long-term conditioning to elevated $pCO_2$ and warming influences the fatty acid and amino acid composition of the diatom *Cylindrotheca fusiformis*. PLoS One **10**, e0123945.

Berner RA. 2004. The Phanerozoic carbon cycle: $CO_2$ and $O_2$. Oxford: Oxford University Press.

Berner RA. 2009. Phanerozoic atmospheric oxygen: new results using the GEOCARBSULF model. American Journal of Science **309**, 603–606.

Bhatti S, Colman B. 2005. Inorganic carbon acquisition by the chrysophyte alga *Mallomonas papillosa.* Canadian Journal of Botany **83**, 891–897.

**Deleted:** Beardall J, Griffiths H, Raven JA. 1982. Carbon isotope discrimination and the $CO_2$ accumulating mechanism in *Chlorella pyrenoidosa.* Journal of Experimental Botany **33**, 729–737.

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☑**Bhatti S, Colman B.** 2008. Inorganic carbon acquisition in some synurophyte algae. Physiologia Plantarum **133**, 33–40.

☑**Bhatti S, Colman B.** 2011. Evidence for the occurrence of photorespiration in synurophyte algae. Photosynthesis Research **109**, 251–256.

☑**Blank CE, Sánchez-Baracaldo P.** 2010. Timing of morphological and ecological innovations in the cyanobacteria—a key to understanding the rise in atmospheric oxygen. Geobiology **8**, 1–23.

☑**Boller AR, Thomas PJ, Cavenaugh M, Scott KM.** 2011. Low stable isotope fractionation by coccolithophore Rubisco. Geochimica et Geophysica Acta **75**, 7200–7207.

☑**Boller AJ, Thomas PJ, Cavanaugh CM, Scott KM.** 2015. Isotopic discrimination and kinetic parameters of RubisCO from the marine bloom-forming diatom, *Skeletonema costatum*. Geobiology **13**, 33–43.

☑**Bombar D, Heller P, Sánchez-Baracaldo P, Carter BJ, Zehr JP.** 2014. Comparative genomics reveals surprising divergence of two closely related strains of uncultivated UCYN-A cyanobacteria. ISME Journal **8**, 2530–2542.

☑**Boyd PW, Cornwall CE, Davison A, Doney SC, Fourquez M, Hurd CL, Lima ID, McMinn A.** 2016*a*. Biological responses to environmental heterogeneity under future ocean conditions. Global Change Biology **22**, 2633–2650.

☑**Boyd PW, Dillingham PW, McGraw CM, *et al*.** 2016*b*. Physiological responses of a Southern Ocean diatom to complex future ocean conditions. Nature Climate Change **6**, 207–213.

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**Breecker DO, Sharp ZD, McFadden LD.** 2010. Atmospheric $CO_2$ concentrations during ancient greenhouse climates were similar to those predicted for AD 2100. Proceedings of the National Academy of Sciences, USA **107**, 575–580.

**Brennan G, Collins S.** 2015. Growth responses of a green alga to multiple environmental drivers. Nature Climate Change **5**, 892–897.

**Brodie J, Williamson CJ, Smale DA, *et al*.** 2014. The future of the northeast Atlantic benthic flora in a high $CO_2$ world. Ecology and Evolution **4**, 2787–2798.

**Brown JW, Sorhanus U.** 2010. A molecular genetic timescale for the diversification of autotrophic stramenopiles (Ochrophyta): substantive underestimation of putative fossil ages. PLoS One **5**, e12759.

**Buchman N, Ehleringer JR.** 1998. $CO_2$ concentration profiles, and carbon and oxygen isotopes in $C_3$ and $C_4$ crop canopies. Agricultural and Forest Meteorology **89**, 45–58.

**Buick R.** 1992. The antiquity of oxygenic photosynthesis: evidence from stromatolites in sulphate-deficient Archaean lakes. Science **255**, 74–77.

**Buick R.** 2008. When did oxygenic photosynthesis evolve? Philosophical Transactions of the Royal Society B: Biological Sciences **363**, 2731–2743.

**Butterfield NJ.** 2000. *Bangiomorpha pubescens* n.gen., n.sp.: implications for the evolution of sex, multicellularity, and mesoproterozoic/neoproterozoic production of eukaryotes. Palaeobiology **26**, 386–404.

**Butterfield NJ.** 2015. Proterozoic photosynthesis—a critical review. Palaeontology **58**, 953–972.

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**Campbell AJ, Waddington ED, Warren SG.** 2014. Refugium for surface life on Snowball Earth in a nearly enclosed sea? A numerical solution for sea-glacier invasion through a narrow strait. Journal of Geophysical Research: Oceans **119**, 2679–2690.

**Campbell L, Vaulot D.** 1993. Photosynthetic picoplankton community structure in the subtropical North Pacific Ocean near Hawaii (Station Aloha). Deep Sea Research Part I Oceanographic Research Papers **48**, 2043–2050.

**Cardona T.** 2015. A fresh look at the evolution and diversification of photochemical reaction centers. Photosynthesis Research **126**, 111–134.

**Casey JR, Lomas MW, Mandecki J, Walker DE.** 2007. *Prochlorococcus* contributes to new production in the Sargasso Sea deep chlorophyll maximum. Geophysical Research Letters **34**, L10604.

**Chi S, Wu S, Wang K, Tang X, Liu T.** 2014. Phylogeny of $C_4$ photosynthesis enzymes based on algal transcriptomic and genomic data supports an archaeal/proteobacterial origin and multiple duplications for most $C_4$ related genes. PLoS One **10**, e110154.

**Chrismas NA, Anesio AM, Sánchez-Baracaldo P.** 2015. Phylogeny and diversity of cyanobacteria from extreme cold environments. Frontiers in Microbiology **6**, 1070.

**Chrismas NA, Barker G, Anesio AM, Sánchez-Baracaldo P.** 2016. Genomic mechanisms for cold tolerance and production of exopolysaccharides in the Arctic cyanobacterium *Phormidesmis priestleyi* BC1401. BMC Genomics **17**, 533.

☑**Claire MW**, **Sheets J**, **Cohen M**, *et al*. 2012. The evolution of solar flux from 0.1 nm to 160 μm: quantitative estimates for planetary studies. Astrophysical Journal **757**, 95.

☑**Clement R**, **Dimnet L**, **Maberly SC**, **Gontero B.** 2016. The nature of the $CO_2$ concentrating mechanisms in a marine diatom, *Thalassiosira pseudonana*. New Phytologist **209**, 1417–1427.

☑**Codispoti LA**, **Friederich GE**, **Hood DW.** 1986. Variability of the inorganic carbon system over the SE Bering Sea during spring 1980 and spring–summer 1981. Continental Shelf Research **5**, 133–160.

☑**Codispoti LA**, **Friederich GE**, **Iverson RL**, **Hood DW.** 1982. Temporal changes in the inorganic carbon system of the south-east Bering Sea during spring 1980. Nature **296**, 242–245.

☑**Collins S**, **Bell G.** 2004. Phenotypic consequences of 1000 generations of selection at elevated $CO_2$ in a green alga. Nature **431**, 566–569.

☑**Collins S**, **Bell G.** 2006. Evolution of natural algal populations at elevated $CO_2$. Ecology Letters **9**, 129–135.

☑**Cornejo-Castillo FM**, **Cabello AM**, **Salazar G**, *et al*. 2016. Cyanobacterial symbionts diverged in the late Cretaceous towards lineage-specific nitrogen fixation factories in single-celled phytoplankton. Nature Communications **7**, 11071.

☑**Crawford RMM.** 2014. Tundra-Taiga biology. Human, plant and animal survival in the Arctic. New York: Oxford University Press.

☑**Delille B**, **Delille D**, **Fiala M**, **Prevost C**, **Frankignoule M.** 2000. Seasonal changes of $pCO_2$ over a subantarctic *Macrocystis* kelp bed. Polar Biology **23**, 706–716.

☑**Dillon JG, Castenholz RW.** 1999. Scytonemin, a cyanobacterial sheath pigment, protects against UVC radiation: implications for early photosynthetic life. Journal of Phycology **35,** 673–681.

☑**Di Rienzi SC, Sharon I, Wrighton KC,** *et al.* 2013. The human gut and groundwater harbor non-photosynthetic bacteria belonging to a new candidate phylum sibling to Cyanobacteria. Elife **1,** e01102.

☑**Doblin MA, van Sebille E.** 2016. Drift in ocean currents impacts intergenerational exposure to temperature. Proceedings of the National Academy of Sciences, USA **113,** 5700–5705.

☑**Doney SC, Ruckelshaus M, Duffy JE,** *et al.* 2012. Climate change impacts on marine ecosystems. Annual Review of Marine Science **4,** 11–37.

☑**Edwards EJ, Still CJ.** 2008. Climate, phylogeny and the ecological distribution of C4 grasses. Ecology Letters **11,** 266–276.

☑**Ehleringer JR, Sage RF, Flanagan LB, Pearcy RW.** 1991. Climate change and the evolution of $C_4$ photosynthesis. Trends in Ecology and Evolution **6,** 95–99.

☑**Eisenhut M, Ruth W, Haimovitch M, Bauwe H, Kaplan A, Hagemann M.** 2008. The photorespiratory glycolate metabolism is essential for cyanobacteria and might have been conveyed endosymbiotically to plants. Proceedings of the National Academy of Sciences, USA **105,** 17199–17204.

☑**Fairchild IJ, Fleming EJ, Bao H,** *et al.* 2015. Continental carbonate facies of a Neoproterozoic panglaciation, north-east Svalbard. Sedimentology **63,** 443–497.

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**Field CB**, **Behrenfeld MJ**, **Randerson JT**, **Falkowski P**. 1998. Primary production of the biosphere: integrating terrestrial and oceanic components. Science **281**, 237–240.

**Finkel ZV**, **Beardall J**, **Flynn KJ**, *et al.* 2010. Phytoplankton in a changing world: cell size and chemical stoichiometry. Journal of Plankton Research **32**, 119–137.

**Fleming ED**, **Prufert-Bebout L**. 2010. Characterization of cyanobacterial communities from high-elevation lakes in the Bolivian Andes. Journal of Geophysical Research Biogeosciences **115**, G00D07.

**Flynn KJ**, **Raven JA**. 2017. What is the limit on photoautotrophic plankton growth rates? Journal of Plankton Research **39**, 13–22.

**Franks PJ**, **Royer DL**, **Beerling DJ**, *et al.* 2014. New constraints on atmospheric $CO_2$ concentration for the Phanerozoic. Geophysical Research Letters **41**, 4685–4694.

**Galmés J**, **Hermida-Carrera C**, **Laanisto L**, **Niinemets Ü**. 2016. A compendium of temperature responses of Rubisco kinetic traits: variability among and within photosynthetic groups and impacts on photosynthesis modeling. Journal of Experimental Botany **67**, 5067–5091.

**Gao K**, **Helbling EW**, **Häder D-P**, **Hutchins DA**. 2012. Responses of marine primary producers to interactions between ocean acidification, solar radiation and warming. Marine Ecology Progress Series **470**, 167–189.

**Genkov T**, **Meyer M**, **Griffiths H**, **Spreitzer RJ**. 2010. Functional hybrid rubisco enzymes with plant small subunits and algal large subunits: engineered rbcS cDNA for expression in *Chlamydomonas*. Journal of Biological Chemistry **285**, 19833–19841.

**Giordano M, Norici A, Forssen M, Eriksson M, Raven JA**. 2003. An anaplerotic role for mitochondrial carbonic anhydrase in *Chlamydomonas reinhardtii*. Plant Physiology **132,** 2126–2134.

**Giordano M, Pezzoni V, Hell R**. 2000. Strategies for the allocation of resources under sulfur limitation in the green alga *Dunaliella salina*. Plant Physiology **124**, 857–864.

**Gonzalez-Esquer CR, Smarda J, Rippka R, Axen SD, Guglielmi G, Gugger M, Kerfeld CA**. 2016. Cyanobacterial ultrastructure in light of genomic sequence data. Photosynthesis Research **129**, 147–157.

**Halevy I, Bachan A.** 2017. The geologic history of seawater pH. Science **355,** 1069-1071.

**Harano K, Ishida H, Kittaka R, Kojima K, Inoue N, Tsukamoto M, Satoh R, Himeno M, Iwaki T, Wadano A**. 2003. Regulation of the expression of ribulose-1,5-bisphosphate carboxylase/oxygenase (EC 4.1.1.39) in a cyanobacterium, *Synechococcus* PCC7942. Photosynthesis Research **78**, 59–65.

**Henley SF, Annett AL, Ganeschram RS,** *et al.* 2012. Factors influencing the stable carbon isotope composition of suspended and sinking organic matter in the coastal Antarctic sea ice environment. Biogeosciences **9,** 1137–1157.

**Hoffman PF.** 2016. Cryoconite pans on Snowball Earth: supraglacial oases for Cryogenian eukaryotes? Geobiology **14,** 531–542.

**Hoffman PF, Schrag DP.** 2002. The snowball Earth hypothesis: testing the limits of global change. Terra Nova **14,** 129–155.

☑**Hofmann HJ.** 1976. Precambrian microflora, Belcher Islands, Canada: significance and systematics. Journal of Paleontology **50,** 1040–1073.

☑**Hopkinson BM, Young JN, Tansik AL, Binder BJ.** 2014. The minimal $CO_2$-concentrating mechanism of *Prochlorococcus* spp. MED4 is effective and efficient. Plant Physiology **166,** 2205–2217.

☑**Horath T, Bachofen R.** 2009. Molecular characterization of an endolithic microbial community in dolomite rock in the central Alps (Switzerland). Microbial Ecology **58,** 290–306.

☑**Hu HH, Zhou QI.** 2010. Regulation of inorganic carbon acquisition by nitrogen and phosphorus levels in *Nannochloropsis* sp. World Journal of Microbiology and Biotechnology **26,** 957–961.

☑**Hutchins DA, Walworth NG, Webb EA, Saito MA, Moran D, McIlvin MR, Gale J, Fu FX.** 2015. Irreversibly increased nitrogen fixation in *Trichodesmium* experimentally adapted to elevated carbon dioxide. Nature Communications **6,** 8155.

☑**Johnson VR, Russell BD, Fabricius KE, Brownlee C, Hall-Spencer JM.** 2012. Temperate and tropical brown macroalgae thrive, despite decalcification, along natural $CO_2$ gradients. Global Change Biology **18,** 2792–2803.

☑**Johnston AM, Kennedy H.** 1998. Carbon stable isotope fractionation in marine stems: open ocean studies and laboratory studies. In: Griffiths H, ed. Stable isotopes—integration of biological ecological and geochemical processes. Oxford: BIOS Scientific Publishers, 293–306.

**Jordan DB, Ogren WL.** 1981. Species variation in the specificity of ribulose bisphosphate carboxylase/oxygenase. Nature **291**, 513–515.

**Kah LC, Riding R.** 2007. Mesoproterozoic carbon dioxide inferred from calcified cyanobacteria. Geology **35**, 799–802.

**Kapralov MV, Filatov DA.** 2007. Widespread positive selection in the photosynthetic Rubisco enzyme. BMC Evolutionary Biology **7**, 73.

**Keeley JE.** 1998. CAM photosynthesis in submerged aquatic plants. Botanical Review **64**, 121–175.

**Keeley JE, Keeley SC.** 1989. Crassulacean acid metabolism (CAM) in high elevation tropical cactus. Plant, Cell and Environment **12**, 331–336.

**Kevekordes K, Holland D, Häubner N, _et al_.** 2006. Inorganic carbon acquisition by eight species of _Caulerpa_ (Caulerpaceae, Chlorophyta). Phycologia **45**, 442–449.

**Koch M, Bowes G, Ross C, Zhang XH.** 2013. Climate change and ocean acidification effects on seagrasses and marine macroalgae. Global Change Biology **19**, 103–132.

**Körner C.** 1999. Alpine plant life. Functional plant ecology of high mountain ecosystems. Berlin: Springer.

**Kozłowska-Szerenos B, Bialuk I, Maleszewski S.** 2004. Enhancement of photosynthetic $O_2$ evolution in _Chlorella vulgaris_ under high light and increased $CO_2$ concentration as a sign of acclimation to phosphate deficiency. Plant Physiology and Biochemistry **42**, 403–409.

**Kranz SA, Young JN, Hopkinson BM, Goldman JA, Tortell PD, Morel FM**. 2015. Low temperature reduces the energetic requirement for the $CO_2$ concentrating mechanism in diatoms. New Phytologist **205**, 192–201.

**Kronholm I, Collins S**. 2016. Epigenetic mutations can both help and hinder adaptive evolution. Molecular Ecology **25**, 1856–1868.

**Ku SB, Edwards GE**. 1977. Oxygen inhibition of photosynthesis: I. Temperature dependence and relation to $O_2/CO_2$ solubility ratio. Plant Physiology **59**, 986–990.

**Kubien DS, Sage RF**. 2003. C4 grasses in boreal fens: their occurrence in relation to microsite characteristics. Oecologia **137**, 330–337.

**Kübler JE, Raven JA.** 1995. The interaction between inorganic carbon supply and light supply in *Palmaria palmata* (Rhodophyta). Journal of Phycology **31**, 369–375.

**Kump LR.** 2008. The rise of atmospheric oxygen. Nature **451**, 277–278.

**Lenton T, Daines S.** 2017. Matworld—the biogeochemical effects of early life on land. New Phytologist (in press).

**Lenton TM, Crouch M, Johnson M, *et al*.** 2012. First plants cooled the Ordovician. Nature Geoscience **5**, 86–89.

**Letelier RM, Karl DM, Abbott MR, Bidigare RR.** 2004. Light driven seasonal patterns of chlorophyll and nitrate in the lower euphotic zone of the North Pacific Subtropical Gyre. Limnology and Oceanography **49**, 508–519.

☑**Li F, Beardall J, Collins S, Gao K.** 2016. Decreased photosynthesis and growth with reduced respiration in the model diatom *Phaeodactylum tricornutum* grown under elevated $CO_2$ over 1800 generations. Global Change Biology **23,** 127–137.

☑**Li W, Gao K, Beardall J.** 2012. Interactive effects of ocean acidification and nitrogen-limitation on the diatom *Phaeodactylum tricornutum*. PLoS One **7,** e51590.

☑**Lohbeck LT, Riebesell U, Reusch TBH.** 2014. Gene expression changes in the coccolithophore *Emiliania huxleyi* after 500 generations of selection to ocean acidification. Proceedings of the Royal Society B: Biological Sciences **281,** 2014003.

☑**Losh JL, Young JN, Morel FM.** 2013. Rubisco is a small fraction of total protein in marine phytoplankton. New Phytologist **198,** 52–58.

☑**Low-Décarie E, Jewell MD, Fussmann GF, Bell G.** 2013. Long-term culture at elevated atmospheric $CO_2$ fails to evoke specific adaptation in seven freshwater phytoplankton species. Proceedings of the Royal Society B: Biological Sciences **280,** 2012590.

☑**Lüttge U.** 2004. Ecophysiology of crassulacean acid metabolism (CAM). Annals of Botany **93,** 629–652.

☑**Lyons TW, Reinhard CT, Planavsky NJ.** 2014. Evolution: a fixed-nitrogen fix in the early ocean? Current Biology **24,** R276–R278.

☑**Maberly SC.** 1996. Diel, episodic and seasonal changes in pH and concentrations of inorganic carbon in a productive lake. Freshwater Biology **35,** 579–598.

**Maberly SC, Ball LA, Raven JA, Sültemeyer D.** 2009. Inorganic carbon acquisition by chrysophytes. Journal of Phycology **45**, 1052–1061.

**Marconi M, Giordano M, Raven JA.** 2011. Impact of taxonomy, geography, and depth on $\delta^{13}C$ and $\delta^{15}N$ variation in a large collection of macroalgae. Journal of Phycology **47**, 1023–1035.

**Mareš J**, **Hrouzek P**, **Kaňa R**, **Ventura S**, **Strunecký O**, **Komárek J.** 2013. The primitive thylakoid-less cyanobacterium *Gloeobacter* is a common rock-dwelling organism. PLoS One **8**, e66323.

**McElwain JC, Montañez I, White JD,** *et al.* 2016. Was atmospheric $CO_2$ capped at 1000 ppm over the last 300 million years? Palaeogeography, Palaeoclimatology, Paleoecology **441**, 653–658.

**Meyer M, Griffiths H.** 2013. Origins and diversity of eukaryotic $CO_2$-concentrating mechanisms: lessons for the future. Journal of Experimental Botany **64**, 769–786.

**Mitchell C, Beardall J.** 1996. Inorganic carbon uptake by an Antarctic sea-ice diatom *Nitzchia frigida*. Polar Biology **16**, 95–99.

**Mock T, Daines SJ, Geider R, Collins S, Metodiev M, Millar AJ, Moulton V, Lenton TM.** 2016. Bridging the gap between omics and earth system science to better understand how environmental change impacts marine microbes. Global Change Biology **22**, 61–75.

**Murphy LR, Barrioca J, Franchschi VR, Lee R, Roalson EH, Edwards GE, Ku MSB.** 2007. Diversity and plasticity of $C_4$ photosynthesis in *Eleocharis* (Cyperaceae). Functional Plant Biology **34**, 571–580.

☑**Nakamura Y, Kaneko T, Sato S,** *et al.* 2003. Complete genome structure of *Gloeobacter violaceus* PCC 7421, a cyanobacterium that lacks thylakoids. DNA Research **10,** 137–145.

☑**Nakayama T, Kamikawa R, Tanifuji G,** *et al.* 2014. Complete genome of a non-photosynthetic cyanobacterium reveals recent adaptations to an intracellular lifestyle. Proceedings of the National Academy of Sciences, USA **111,** 11407–11412.

☑**Namsaraev Z, Mano NJ, Fernandez R, Wilmotte A.** 2010. Biogeography of terrestrial cyanobacteria from Antarctic ice-free areas. Annals of Glaciology **51,** 171–177.

☑**Nienow JA, McKay CP, Friedmann EI.** 1988. The cryptoendolithic microbial environment in the Ross Desert of Antarctica: light in the photosynthetically active region. Microbial Ecology **16,** 271–289.

☑**Oehler DZ.** 1977. Pyrenoid-like structures in the late *Precambrian algae* from the bitter springs formation in Australia. Journal of Palaeontology **51,** 885–901.

☑**Olcott AN, Sessions AL, Corsetti FA, Kaufman AJ, de Oliviera TF.** 2005. Biomarker evidence for photosynthesis during neoproterozoic glaciation. Science **310,** 471–474.

☑**Olson SL, Reinhard CT, Lyons TW.** 2016. A limited role for methane in the mid-Proterozoic greenhouse. Proceedings of the National Academy of Sciences, USA **113,** 11447–11452.

☑**Olsson-Francis K, de la Torre R, Cockell CS.** 2010. Isolation of novel extreme-tolerant cyanobacteria from a rock-dwelling microbial community by using

exposure to low Earth orbit. Applied and Environmental Microbiology **76**, 2115–2121.

☑**Padfield D**, **Yvon-Durocher G**, **Buckling A**, ***et al***. 2016. Rapid evolution of metabolic traits explains thermal adaptation in phytoplankton. Ecology Letters **19**, 133–142.

☑**Pittermann J**, **Sage RF**. 2001. The response of the high altitude $C_4$ grass *Muhlenbergia montana* (Nutt.) A.S. Hitchc. to long- and short-term chilling. Journal of Experimental Botany **52**, 829–838.

☑**Porzio L**, **Buia MC**, **Hall-Spencer JM**. 2011. Effects of ocean acidification on macroalgal communities. Journal of Experimental Marine Biology and Ecology **400**, 278–287.

☑**Porzio L**, **Garrard SL**, **Buia MC**, **Hall-Spencer JM**. 2013. The effects of ocean acidification on early algal colonization stages at natural $CO_2$ vents. Marine Biology **160**, 2247–2259.

☑**Price GD**, **Badger MR**. 1989*a*. Expression of human carbonic anhydrase in the cyanobacterium *Synechococcus* PCC7942 creates a high $CO_2$-requiring phenotype: evidence for a central role for carboxysomes in the $CO_2$ concentrating mechanism. Plant Physiology **91**, 505–513.

☑**Price GD**, **Badger MR**. 1989*b*. Isolation and characterization of high $CO_2$-requiring-mutants of the cyanobacterium *Synechococcus* PCC7942: two phenotypes that accumulate inorganic carbon but are apparently unable to generate $CO_2$ within the carboxysome. Plant Physiology **91**, 514–525.

☑**Price GD**, **Badger MR**, **Woodger FJ**, **Long BM**. 2008. Advances in understanding the cyanobacterial $CO_2$-concentrating-mechanism (CCM): functional components,

Ci transporters, diversity, genetic regulation and prospects for engineering into plants. Journal of Experimental Botany **59**, 1441–1461.

☑**Pyankov VI, Black CC, Artyuschera EG, Vosnesenskaya EV, Ku MSB, Edwards GE.** 1990. Features of photosynthesis in *Haloxylon* species of the Chenopodiaceae that are dominant plants in central Asian deserts. Plant and Cell Physiology **40**, 125–134.

☑**Pyankov VI, Voznesenskaya EV, Kuz'min AN, Ku MS, Ganko E, Franceschi VR, Black CC** Jr, **Edwards GE.** 2000. Occurrence of C$_3$ and C$_4$ photosynthesis in cotyledons and leaves of *Salsola* species (Chenopodiaceae). Photosynthesis Research **63**, 69–84.

☑**Raven JA.** 1997. The role of marine biota in the evolution of terrestrial biota: gases and genes. Biogeochemistry **39**, 139–164.

☑**Raven JA.** 2009. Contributions of anoxygenic and oxygenic photolithotrophy and chemolithotrophy.to carbon and oxygen fluxes in aquatic systems. Aquatic Microbial Ecology **56**, 177–192.

☑**Raven JA.** 2013. Rubisco: still the most abundant protein of Earth? New Phytologist **198**, 1–3.

☑**Raven JA, Ball LA, Beardall J, *et al.*** 2005. Algae lacking carbon-concentrating mechanisms. Canadian Journal of Botany **83,** 879–890.

☑**Raven JA, Beardall J.** 2014. CO$_2$ concentrating mechanisms and environmental change. Aquatic Botany **118**, 24–37.

**Raven JA, Beardall J.** 2016*a*. Algal photosynthesis and physiology. In: Slocombe SP, Benemann JR, eds. Microalgal production for biomass and high value products. Boca Raton, FL: CRC Press (Taylor and Francis Group), 1–19.

**Raven JA, Beardall J.** 2016*b*. The ins and outs of CO$_2$. Journal of Experimental Botany **67**, 1–13.

**Raven JA, Giordano M.** 2017. Acquisition and metabolism of carbon in the Ochrophyta other than diatoms. Philosophical Transactions of the Royal Society B: Biological Sciences (in press)

**Raven JA, Giordano M, Beardall J, Maberly SC.** 2012. Algal evolution in relation to atmospheric CO$_2$: carboxylases, carbon-concentrating mechanisms and carbon oxidation cycles. Philosophical Transactions of the Royal Society B: Biological Sciences **367**, 493–507.

**Raven JA, Johnston AM, Kübler JE,** *et al*. 2002*a*. Mechanistic interpretation of carbon isotope discrimination by marine macroalgae and seagrasses. Functional Plant Biology **29**, 355–378.

**Raven JA, Johnston AM, Kübler JE,** *et al*. 2002*b*. Seaweeds in cold seas: evolution and carbon acquisition. Annals of Botany **90**, 525–536.

**Raven JA, Kübler JE, Beardall J.** 2000. Put out the light, and then put out the light. Journal of the Marine Biological Association of the UK **80**, 1–25.

**Retallack GJ, Gose BN, Osterhout JI.** 2015. Periglacial paleosols and Cryogenian paleoclimate near Adelaide South Australia. Precambrian Research **263**, 1–18.

**Reusch TB, Boyd PW.** 2013. Experimental evolution meets marine phytoplankton. Evolution **67**, 1849–1859.

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☑**Raven JA, Giordano M, Beardall J, Maberly SC.** 2012. Algal evolution in relation to atmospheric CO$_2$: carboxylases, carbon-concentrating mechanisms and carbon oxidation cycles. Philosophical transactions of the Royal Society of London. Series B **367**, 493–507.¶

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☑**Riding R.** 2006. Cyanobacterial calcification, carbon dioxide concentrating mechanisms and Proterozoic–Cambrian changes in atmospheric composition. Geobiology **4**, 283–298.

☑**Royer DL.** 2014. Atmospheric $CO_2$ and $O_2$ during the Phanorozoic: tools, patterns and impacts. In: Treatise on geochemistry, 2nd edn, Vol. 6. Amsterdam: Elsevier, 251–267.

☒**Ruan Z, Giordano M, Raven JA.** 2017. Energy partitioning between N assimilation and C acquisition and assimilation in energy and carbon-limited *Synechococcus* species. Journal of Experimental Botany, **68** (in press).

☑**Runcie JW, Gurgel CFD, McDermid KJ.** 2008. In situ photosynthetic rates of tropical marine macroalgae at their lower depth limit. European Journal of Phycology **43**, 377–388.

☑**Sánchez-Baracaldo P.** 2015. Origin of marine planktonic cyanobacteria. Scientific Reports **5**, 17418.

☑**Sánchez-Baracaldo P, Ridgwell A, Raven JA.** 2014. A neoproterozoic transition in the marine nitrogen cycle. Current Biology **24**, 652–657.

☑**Saw JH, Schatz M, Brown MV, Kunkel DD, Foster JS, Shick H, Christensen S, Hou S, Wan X, Donachie SP.** 2013. Cultivation and complete genome sequencing of *Gloeobacter kilaueensis* sp. nov., from a lava cave in Kīlauea Caldera, Hawai'i. PLoS One **8**, e76376.

☑**Saxby-Rouen KJ, Leadbeater BSC, Reynolds CS.** 1997. The growth response of *Synura petersenii* (Synurophyceae) to photon flux density, temperature and pH. Phycologia **36**, 233–243.

☑**Saxby-Rouen KJ, Leadbeater BSC, Reynolds CS.** 1998. The relationship between growth of *Synura petersenii* (Synurophyceae) and components of the dissolved inorganic carbon system. Phycologia **37**, 467–477.

☑**Schaum CE, Collins S.** 2014. Plasticity predicts evolution in a marine alga. Proceedings of the Royal Society B: Biological Sciences **281**, 20141486.

☑**Schaum CE, Rost B, Collins S.** 2016. Environmental stability affects phenotypic evolution in a globally distributed marine picoplankton. ISME Journal **10**, 75–84.

☑**Scheinin M, Riebesell U, Rynearson TA,** *et al.* 2015. Experimental evolution gone wild. Journal of the Royal Society Interface **12**, 20150056.

☑**Schirrmeister BE, Gugger M, Donoghue PC.** 2015. Cyanobacteria and the great oxidation event: evidence from genes and fossils. Palaeontology **58**, 769–785.

☑**Schirrmeister BE, Sánchez-Baracaldo P, Wacey D.** 2016. Cyanobacterial evolution during the Precambrian. International Journal of Astrobiology **15,** 187–204.

☑**Schlüter L, Lohbeck KT, Gröger JP, Riebesell U, Reusch TB.** 2016. Long-term dynamics of adaptive evolution in a globally important phytoplankton species to ocean acidification. Science Advances **2,** e1501660.

☑**Schlüter L, Lohbeck KT, Gutowska MA,** *et al.* 2014. Adaptation of a globally important coccolithophore to ocean warming and acidification. Nature Climate Change **4**, 1024–1030.

☑**Scott KM, Henn-Sax M, Harmer YL,** *et al.* 2007. Kinetic isotope effect and biochemical characterization of Form IA RubisCO from the marine cyanobacterium *Prochlorococcus marinus* MIT9313. Limnology and Oceanography **52**, 2199–2204.

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☑**Sharkey TD, Berry JA.** 1985. Carbon dioxide fractional of algae as influenced by an inducible $CO_2$ concentrating mechanism. In: Lucas WJ, Berry JA, eds. Inorganic carbon uptake by aquatic organisms. Rockville, MD: American Society of Plant Physiologists, 389–401.

☑**Shih PM, Occhialini A, Cameron JC, Andralojc PJ, Parry MA, Kerfeld CA.** 2016. Biochemical characterization of predicted Precambrian RuBisCO. Nature Communications **7**, 10382.

☑**Smith RC, Marra J, Perry MJ, *et al*.** 1989. Estimation of photon flux for the upper ocean in the Sargasso Sea. Limnology and Oceanography **34**, 1675–1693.

☑**Som SM, Buick R, Hagadorn JW, *et al*.** 2016. Earth's air pressure 2.7 billion years ago constrained to less than half of present levels. Nature Geosciences **9**, 448–451.

☑**Soo RM, Skennerton CT, Sekiguchi Y, Imelfort M, Paech SJ, Dennis PG, Steen JA, Parks DH, Tyson GW, Hugenholtz P.** 2014. An expanded genomic representation of the phylum cyanobacteria. Genome Biology and Evolution **6**, 1031–1045.

☑**Spijkerman E, Stojkovic S, Beardall J.** 2014. $CO_2$ acquisition in *Chlamydomonas acidophila* is influenced mainly by $CO_2$, not phosphorus, availability. Photosynthesis Research **121**, 213–221.

☑**Stepien CC.** 2015. Impacts of geography, taxonomy and functional group on inorganic carbon use patterns in marine macrophytes. Journal of Ecology **103**, 1372–1383.

☑**Stepien CC, Pfister CA, Wootton JT.** 2016. Functional traits for carbon access in macrophytes. PLoS One **11**, e0159062.

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**Still CJ, Berry JA, Collatz GJ, DeFries RS.** 2003. Global distribution of $C_3$ and $C_4$ vegetation. Global Biogeochemical Cycles **17**, 1006.

**Strother PK, Battison L, Brasier MD, Wellman CH.** 2011. Earth's earliest non-marine eukaryotes. Nature **473**, 505–509.

**Tcherkez GGB, Farquahar GD, Andrews TJ.** 2006. Despite slow catalysis and confused substrate specificities, all ribulose bisphosphate carboxylases may be nearly perfectly optimized. Proceedings of the National Academy of Sciences, USA **103**, 7246–7251.

**Trimborn S, Brenneis T, Sweet E, Rost B.** 2013. Sensitivity of Antarctic phytoplankton species to ocean acidification. Growth, carbon acquisition and species interaction. Limnology and Oceanography **58**, 997–1007.

**Uemura K, Anwaruzzaman, Miyachi S, Yokota A.** 1997. Ribulose-1,5-bisphosphate carboxylase/oxygenase from thermophilic red algae with a strong specificity for $CO_2$ fixation. Biochemical and Biophysical Research Communications **233**, 568–571.

**Ueno O, Samejima M, Muto S, Miyachi S.** 1988. Photosynthetic characteristics of an amphibious plant, *Eleocharis vivipara*: expression of $C_4$ and $C_3$ modes in contrasting environments. Proceedings of the National Academy of Sciences, USA **85**, 571–580.

**Villareal JC, Renner SS.** 2012. Hornwort pyrenoids, carbon-concentrating structures, evolved and were lost at least five times in the last 100 million years. Proceedings of the National Academy of Sciences, USA **109**, 18873–19978.

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**Vincent WF, Gibson JA, Pienitz R, Villeneuve V, Broady PA, Hamilton PB, Howard-Williams C.** 2000. Ice shelf microbial ecosystems in the high arctic and implications for life on snowball earth. Die Naturwissenschaften **87**, 137–141.

**Wacey D, Brasier M, Parnell J, et al.** 2016. Contrasting microfossil preservation and lake chemistry with the 1200–1000 Ma Torridian supergroup of NW Scotland. In: Brasier AT, McInroy D, McLoughlin, eds. Earth system evolution and early life: a celebration of the work of Martin Brasier. London: Geological Society, Special Publication, 448.

**Wallworth NG, Lee MD, Fu F-X, et al.** 2016. Molecular and physiological evidence of genetic assimilation to high $CO_2$ in the marine nitrogen fixer *Trichodesmium*. Proceedings of the National Academy of Sciences, USA **113**, E7367–E7374.

**Wang L, Yamano T, Kajikawa M, Hirono M, Fukuzawa H.** 2014. Isolation and characterization of novel high-$CO_2$-requiring mutants of *Chlamydomonas reinhardtii*. Photosynthesis Research **121**, 175–184.

**Wellman CH, Strother PK.** 2015. The terrestrial biota prior to the origin of land plants (Embryophytes): a review of the evidence. Palaentology **58**, 6501–627.

**Wu Z, Zend B, Li R, Song L.** 2012. Combined effects of carbon and phosphorus on the invasive cyanobacterium *Cylindrospermopsis raciborski*. Phycologia **51**, 144–150.

**Xu Z, Gao K.** 2009. Impacts of UV radiation on growth and photosynthetic carbon assimilation in *Gracilaria lemaneiformis* (Rhodophyta) under phosphorus limited and replete conditions. Functional Plant Biology **36**, 1057–1064.

☑**Xu Z, Zou D, Gao K.** 2010. Effects of elevated $CO_2$ and phosphorus supply on growth, photosynthesis and nutrient uptake in the marine macroalga *Gracilaria lemaneiformis* (Rhodophyta). Botanica Marina **53**, 123–129.

☑**Yang EC, Boo GH, Kim HJ, Cho SM, Boo SM, Andersen RA, Yoon HS.** 2012. Supermatrix data highlight the phylogenetic relationships of photosynthetic stramenopiles. Protist **163**, 217–231.

☑**Ye Q, Tong J, Zhu S, An J.** 2015. The survival of benthic macroscopic phototrophs on a neoproterozoic snowball earth. Geology **43**, 507–510.

☑**Yokota A, Iwaki T, Miura K, *et al*.** 1987. Model for the relationship between $CO_2$-concentrating mechanism, $CO_2$ fixation, and glycolate synthesis during photosynthesis in *Chlamydomonas reinhardtii*. Plant and Cell Physiology **28**, 1363–1376.

☑**Young EB, Beardall J.** 2005. Modulation of photosynthesis and inorganic carbon acquisition in a marine microalga by nitrogen, iron and light availability. Canadian Journal of Botany **83**, 917–928.

☑**Young JN, Goldman JA, Kranz SA, Tortell PD, Morel FM.** 2015*b*. Slow carboxylation of Rubisco constrains the rate of carbon fixation during *Antarctic phytoplankton* blooms. New Phytologist **205,** 172–181.

☑**Young JN, Heureux AM, Sharwood RE, Rickaby RE, Morel FM, Whitney SM.** 2016. Large variation in the Rubisco kinetics of diatoms reveals diversity among their carbon-concentrating mechanisms. Journal of Experimental Botany **67,** 3445–3456.

☑**Young JN, Kranz SA, Goldman JAL, Tortell PD, Morel FMM.** 2015*a*. Antarctic phytoplankton down-regulate their carbon-concentrating mechanisms under high $CO_2$ with no change in growth rates. Marine Ecology Progress Series **532**, 13–28.

☑**Young JN, Rickaby RE, Kapralov MV, Filatov DA.** 2012. Adaptive signals in algal Rubisco reveal a history of ancient atmospheric carbon dioxide. Philosophical Transactions of the Royal Society B: Biological Sciences **367**, 483–492.

☑**Zehr JP, Bench SR, Carter BJ, Hewson I, Niazi F, Shi T, Tripp HJ, Affourtit JP.** 2008. Globally distributed uncultivated oceanic $N_2$-fixing cyanobacteria lack oxygenic photosystem II. Science **322**, 1110–1112.

**Table 1.** Effects of altitude and latitude as general proxies for low temperature, for oxygenic photosynthetic organisms with and without CCMs

| Organisms | Altitude | Latitude | References |
|---|---|---|---|
| $C_3$ terrestrial flowering plants | 6400 m | 83°N | Körner (1999); Crawford (2014) |
| $C_4$ terrestrial flowering plants | 3300 m | 60°N | Pitterman and Sage (2001); Kubien and Sage (2003); Still *et al.* (2003) |
| CAM terrestrial flowering plants | 4700 m | 50°N | Keeley and Keeley (1989); Lüttge (2004) |
| CAM freshwater tracheophytes | 4750 m | 61°N | Keeley (1998) |
| Marine planktonic algae | Not applicable | Increasing contribution of algae lacking CCMs at high southern latitudes | Johnston and Kennedy (1998); Henley *et al.* (2012); Trimborn *et al.* 2015; Young *et al.* (2015) |
| Marine macroalgae | Not applicable | Increasing fraction of algae lacking CCMs at higher latitudes for Phaeophyceae and | Stepien (2015) |

| | | Rhodophyta, not Chlorophyta | |
|---|---|---|---|
| Inland water and terrestrial cyanobacteria (CCMs constitutive in photosynthetically competent cyanobacteria) | 5997 m (volcanic area; possibly higher temperatures than other tropical sites at this altitude) | 87°S (Antarctic dry valleys) | Fleming *et al.* (2010); Namsernaev *et al.* (2010) |

**Table 2.** Effects of limitation of growth by the availability of N ($NH_4^+$), N ($NO_3^-$, P, Fe, and S on CCMs as indicated by the half-saturation value for $CO_2$ ($K_{0.5}$), inorganic carbon concentration factor (CCF), and natural abundance $^{13}C{:}^{12}C$ stable isotope of organic matter relative to source $CO_2$ ($\Delta^{13}C$)

| Organism | Limiting nutrient | Effect of limitation on CCM activity | Basis of CCM determination | References |
|---|---|---|---|---|
| *Chlorella emersonii* (Trebouxiophyceae) | N ($NH_4^+$) | Increase | CCF, $K_{0.5}$, $\Delta^{13}C$ | Beardall *et al.* (1982) |
| *Chlorella emersonii* (Trebouxiophyceae) | N ($NO_3^-$) | Increase (only when μ <10% $\mu_{max}$) | $K_{0.5}$ | Beardall *et al.* (1991) |
| *Chlamydomonas reinhardtii* (Chlorophyceae) | N ($NH_4^+$) | Decrease | $K_{0.5}$ | Giordano *et al.* (2003) |
| *Dunaliella tertiolecta* (Chlorophyceae) | N ($NO_3^-$) | Increase | $K_{0.5}$ | Young and Beardall (2005) |
| *Nannochloropsis* sp. (Eustigmatophyceae) | N ($NO_3^-$) | Increase at moderate limitation, but no difference compared with an N-replete control at greater limitation | $K_{0.5}$ | Hu and Zhou (2010) |
| *Phaeodactylum tricornutum* (Bacillariophyceae) | N ($NO_3^-$) | Decrease under N limitation in cells grown in air-equilibrium $CO_2$; similarly decreased values in both replete and limited | $K_{0.5}$ | Li *et al.* (2012) |

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| Species | Nutrient | Effect | Measure | Reference |
|---|---|---|---|---|
| | | cells at elevated (1000 µmol mol$^{-1}$) $CO_2$ | | |
| *Synechococcus* sp. UTEX LB2380 | N ($NH_4^+$, $NO_3^-$) | CCF higher in $NH_4^+$-grown than $NO_3^-$-grown cells under light limitation and repletion. Lower $CO_2$ affinity for $NO_3^-$ cells only at low light | CCF, $K_{0.5}$ | Ruan *et al.* (2017) |
| *Chlorella vulgaris* (Trebouxiophyceae) | P | Increase | $K_{0.5}$ | Kozłowska-Szerenos *et al.* (2004) |
| *Chlorella emersonii* (Trebouxiophyceae) | P | Decrease | $K_{0.5}$, $\Delta^{13}C$ CCF | Beardall *et al.* (2005) |
| *Chlamydomonas acidophila* (Chlorophyceae) | P | Increase | $K_{0.5}$, CCF | Spijkerman *et al.* 2014 |
| *Cylindrospermopsis raciborskii* (Cyanobacterium) | P | Increase | $K_{0.5}$ | Wu *et al.* (2012) |
| *Gracilaria lemanaeiformis* (Florididiophyceae | P | Decrease | $K_{0.5}$ | Xu and Gao (2009); Xu *et al.* (2010) |
| *Nannochlopsis* sp. (Eustigmatophyceae) | P | Decrease | $K_{0.5}$ | Hu and Zhou (2010) |
| *Dunaliella tertiolecta* (Chlorophyceae) | Fe | Increase | $K_{0.5}$, CCF | Young and Beardall 2005 |
| *Dunaliella salina* (Chlorophyceae) | S | Decrease | $K_{0.5}$ | Giordano *et al.*, 2000 |

**Fig. 1.** Time line of major geological eras, showing glaciation events referred to in the text. Within the Phanerozoic, eras are: Cen, Cenozoic; Mes, Mesozoic; Pal, Paleozoic. For the Archean and Proterozoic, Paleo, Meso and Neo are prefixes for the eras.

**Fig. 2.** Estimates of past $CO_2$ in µmol mol$^{-1}$ total gas. Data were abstracted from Breecker *et al.* (2010), Kah and Riding (2007), and Royer (2014).

**Fig. 3.** Estimates of past $O_2$ in kPa partial pressure. Data were abstracted from Berner (2009), Kump (2008), and Royer (2014).

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