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COMMENTARY

The ins and outs of CO_2

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Abstract

It is difficult to distinguish influx and efflux of inorganic C in photosynthesizing tissues; this article examines what is known and where there are gaps in knowledge. Irreversible decarboxylases produce CO_2, and CO_2 is the substrate/product of enzymes that act as carboxylases and decarboxylases. Some irreversible carboxylases use CO_2; others use HCO_3^-. The relative role of permeation through the lipid bilayer versus movement through CO_2-selective membrane proteins in the downhill, non-energized, movement of CO_2 is not clear. Passive permeation explains most CO_2 entry, including terrestrial and aquatic organisms with C_3 physiology and biochemistry, terrestrial C_4 plants and all crassulacean acid metabolism (CAM) plants, as well as being part of some mechanisms of HCO_3^- use in CO_2 concentrating mechanism (CCM) function, although further work is needed to test the mechanism in some cases. However, there is some evidence of active CO_2 influx at the plasmalemma of algae. HCO_3^- active influx at the plasmalemma underlies all cyanobacterial and some algal CCMs. HCO_3^- can also enter some algal chloroplasts, probably as part of a CCM. The high intracellular CO_2 and HCO_3^- pools consequent upon CCMs result in leakage involving CO_2, and occasionally HCO_3^-. Leakage from cyanobacterial and microalgal CCMs involves up to half, but sometimes more, of the gross inorganic C entering in the CCM; leakage from terrestrial C_4 plants is lower in most environments. Little is known of leakage from other organisms with CCMs, though given the leakage better-examined organisms, leakage occurs and increases the energetic cost of net carbon assimilation.

Key words: Aquaporins, bicarbonate, carbon concentrating mechanisms, C_4, carbon dioxide, crassulacean acid metabolism, leakage, lipid bilayer, permeability.

Introduction

The textbook equations for oxygenic photosynthesis and for dark respiration have CO_2 as, respectively, the inorganic C substrate and the inorganic C product. This is the case for the core autotrophic carboxylase of oxygenic photosynthetic organisms, ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco), with CO_2 as its inorganic carbon substrate, and for the decarboxylases of dark respiration with CO_2 as their inorganic C product (Raven, 1972a; Table 5.2 of Raven, 1984; Table 3 of Raven, 1997a). Add to this the Overton prediction over a century ago that CO_2 has a high permeability in lipid bilayers (see Endeward *et al.*, 2014) and it appears at first sight that the textbook equations describe not just the inorganic

C substrate for photosynthesis and inorganic C product of dark respiration, but also the inorganic C species crossing cell membranes between the external environments and intracellular sites of inorganic C consumption and production.

However, it is clear that this picture is significantly oversimplified in (at least) two ways. One is that we now know of CO_2-permeable channels (a subset of the aquaporins, and analogues) in some cell membranes, and there is considerable debate as to their functional significance if the CO_2 permeability of the lipid bilayer is very high (Boron *et al.*, 2011; Itel *et al.*, 2012; Endeward *et al.*, 2014; Kai and Kaldendorf, 2014). The second over-simplification is that there are known CO_2 concentrating mechanisms (CCMs), involving active transport of some inorganic C species (or H^+) and/or C_4 or crassulacean acid metabolism (CAM) biochemistry, accounting for about half of global primary productivity. This assertion is based on the global net primary productivity values of 56 Pg C per year on land and 49 Pg C per year in the ocean (Field *et al.*, 1998), the assumption that the ratio of global C_4 gross primary productivity (almost all terrestrial) to total gross primary productivity, i.e. 0.23 (Still and Berry, 2003), also applies to net primary productivity, and the assumption that not less than 0.8 of the marine global net primary productivity is carried out by organisms with CCMs (Raven *et al.* 2012, 2014; Raven and Beardall, 2014). These assumptions give a total CCM-based global net primary productivity of $(0.23 \times 56) + (0.8 \times 49)$ or 52 Pg C per year out of a total of 105 Pg C per year global net primary productivity.

CCMs necessarily involve an energy input to generate a net flux of inorganic C from the environment with a relatively low CO_2 concentration to the active site of Rubisco where a higher steady-state CO_2 concentration is maintained during photosynthesis. This means that the direction of the CO_2 free energy gradient (inside concentration > outside) is the opposite of that for photosynthesis with C_3 physiology and biochemistry (inside < outside). Accordingly, in an organism expressing a CCM, the high CO_2 permeability of the pathway from the environment to Rubisco required for high rates of photosynthesis in organisms with C_3 physiology and biochemistry would result in a decreased net rate of photosynthesis and increased energy requirement per net CO_2 assimilated (Raven *et al.*, 2014). The final result is an increased energy cost of CCMs relative to that of diffusive entry with C_3 physiology and biochemistry.

Important progress in understanding bidirectional fluxes of inorganic carbon in an organism expressing a CCM has been made in a recent paper in the *Journal of Experimental Botany* (Eichner *et al.*, 2015). Using the marine diazotrophic cyanobacterium *Trichodesmium*, this work combined two experimental approaches, membrane inlet mass spectrometry to distinguish CO_2 from HCO_3^- fluxes (Badger *et al.*, 1994) and measurements of the natural abundance of ^{13}C relative to ^{12}C (Sharkey and Berry, 1985), with modelling. A very important conclusion is that internal cycling of inorganic C is significant for the natural isotope abundance of ^{13}C:^{12}C in the organism, and for cellular energy budgets. This commentary considers wider aspects of CCMs and of leakage of inorganic carbon from them, and how the findings of Eichner *et al.* (2015) might help further interpretation of data on other organisms, including eukaryotic algae and vascular plants.

Species of inorganic C involved in carboxylases and decarboxylases

All of the unidirectional decarboxylases examined (functioning far from thermodynamic equilibrium), i.e. those of the tricarboxylic acid cycle and the oxidative pentose phosphate pathway, produce CO_2 (Raven, 1972a,b). By analogy with such unidirectional decarboxylases, the product of glycine decarboxylase, the enzyme responsible for CO_2 production in the photorespiratory carbon oxidation cycle, is also very likely to be CO_2.

Enzymes that function *in vivo* sufficiently close to thermodynamic equilibrium, and hence can function as carboxylases and decarboxylases, both consume and produce CO_2 (Table 5.2 of Raven, 1984; Häusler *et al.*, 1987; Jenkins *et al.*, 1987; Table 3 of Raven, 1997a). Significantly for the present article, the decarboxylase function of three of these enzymes (phosphoenolpyruvate carboxykinase; NAD^+ malic enzyme; $NADP^+$ malic enzyme) is involved in the decarboxylation step of C_4 and CAM photosynthesis (Jenkins *et al.*, 1987).

Among unidirectional carboxylases, operating far from thermodynamic equilibrium, a number use CO_2 as the inorganic C substrate (Table 5.2 of Raven, 1984; Häusler *et al.*, 1987; Jenkins *et al.*, 1987; Table 3 of Raven, 1997a; Firestyne *et al.*, 2009). Importantly for the present article, these CO_2-consuming carboxylases include Rubisco, the core carboxylase of all oxygenic photosynthetic organisms, as well as the 5-aminoimidazole ribonucleotide carboxylase required for purine synthesis.

Finally, some unidirectional carboxylases consume HCO_3^- (Table 5.2 of Raven, 1984; Table 3 of Raven, 1997a). One of these is phosphoenolpyruvate carboxylase, an essential anaplerotic enzyme in almost all oxygenic photosynthetic organisms (Table 4 of Raven, 1997a) as well as the 'C_3 + C_1' carboxylase of organisms with C_4 photosynthesis (with the exception of the ulvophycean marine macroalga *Udotea flabellum*: see Raven, 1997a) and with CAM photosynthesis. A possible alternative 'C_3 + C_1' carboxylase for C_4 and CAM photosynthesis is pyruvate carboxylase, which also uses HCO_3^- as the inorganic C substrate (Table 5.2 of Raven, 1984; Table 3 of Raven, 1997a). Other carboxylases using HCO_3^- include acetyl CoA carboxylase used in the synthesis of long-chain fatty acids, and carbamoyl phosphate synthase, essential for citrulline, and hence arginine, synthesis (Table 4 of Raven, 1984; Table 3 of Raven, 1997a).

CO_2 permeability of lipid bilayers and the role of CO_2-conducting aquaporins and analogous protein pores

There is still significant uncertainty as to the mechanism of CO_2 permeation of biological membranes (Boron *et al.*, 2011; Itel *et al.*, 2012; Endeward *et al.*, 2014; Kai and Kaldendorf, 2014).

A particular problem is the role of proteinaceous CO_2 channels if the intrinsic CO_2 permeability of the lipid bilayer is very high, although there is evidence of increased photosynthesis and growth in terrestrial C_3 plants expressing CO_2-transporting aquaporins (Uehlein *et al.*, 2003, 2008; Heckwolf *et al.*, 2011) The most convincing evidence for the role of aquaporins in terrestrial C_3 plants comes from Hanba *et al.* (2004) and Tsuchihira *et al.* (2010). Table 1 shows the permeability coefficient for CO_2 of planar lipid bilayers of various compositions, and for the plasmalemma vesicles derived from high and low CO_2-grown *Chlamydomonas reinhardtii.* In all three cases attempts were made to eliminate the influence of diffusion boundary layers on each side of the membrane on the measured permeability.

CO_2 entry in organisms lacking a biophysical CCM

The classic example of these is the C_3 vascular land plants. It is now clear that CO_2 is the species of inorganic carbon entering the cells from the cell wall (Colman and Espie, 1985; Espie and Colman, 1986; Espie *et al.*, 1986; Evans *et al.*, 2009; Maberly, 2014). The assumption is that terrestrial C_4 and CAM vascular plants also rely on CO_2 entry from the cell wall to the cytosol where carbonic anhydrase equilibrates CO_2 with HCO_3^-, the inorganic C substrate for PEPc (Colman and Espie, 1985; Nelson *et al.*, 2005).

For C_3 plants the transport of CO_2 from the outside of the cell wall to Rubisco involves diffusion of CO_2 across the plasmalemma and across the outer and inner chloroplast membranes and, in the aqueous phase, through the cell wall, the cytosol and the stroma (Colman and Espie, 1985). It is implicitly assumed that there is no carbonic anhydrase in the cell wall (Raven and Glidewell, 1981; Colman and Espie, 1985) of C_3 plants, though there seems to be no experimental evidence demonstrating this. Carbonic anhydrases could equilibrate CO_2 and HCO_3^- in the cytosol and stroma and so enlist the predominant (at the pH of the cytosol and stroma) inorganic species, HCO_3^-, in CO_2 transport across these aqueous phases, with the required H^+ flux carried inwards by protonated buffers with a return flux outwards of the deprotonated buffers (Raven and Glidewell, 1981; Colman and Espie, 1985;

Table 1. *Permeability coefficient for CO_2 in planar lipid bilayers and plasmalemma vesicles, corrected as far as possible for limitation by aqueous diffusion boundary layers*

Also shown are the modelled 'optimum' or 'maximum' (for functioning in the CCM) CO_2 permeability of the wall of cyanobacterial carboxysomes and/or the estimated CO_2 permeability of the wall of cyanobacterial carboxysomes.

Experimental system	CO_2 permeability m s^{-1}	References
Planar lipid bilayer composed of 1:1 egg lecithin:cholesterol. 22–24 °C	$3.5 \pm 0.4.10^{-3}$ (standard error)	Gutknecht *et al.* (1977)
Plasmalemma vesicles of *C. reinhardtii* grown photolithtrophically in media with high low (350 µmol mol^{-1} total gas) and high (50 mmol mol^{-1} total gas) CO_2 for growth.? °C	0.76 ± 0.03–$1.49 \pm 0.2.10^{-5}$ (± standard error; low CO_2-grown cells) 1.21 ± 0.01–$1.8 \pm 0.17.10^{-5}$ (± standard error; high CO_2-grown cells)	Sültemeyer and Rinast (1996)
Planar lipid bilayer composed of (i) pure diphytanoyl-phosphatidyl choline (ii) 3:2:1 cholesterol: diphytanoyl-phosphatidyl choline: egg sphingomyelin, and (iii) mixture of lipids mimicking the red cell plasmalemma.? °C	$\geq 3.2 \pm 1.6.10^{-2}$(not clear what ± refers to; ≥3.2 refers to all three membrane compositions)	Missner *et al.* (2008)
Estimate of upper limit on CO_2 permeability of cyanobacterial carboxysome wall consistent with CCM function.	10^{-7}–$2.5.10^{-6}$	Reinhold et al. (1987, 1991)
Estimate of CO_2 permeability of the carboxysome wall of *Synechococcus* assuming all of the limitation of CO_2 efflux from carboxysomes is in the carboxysome wall. 30 °C	$2.2.10^{-7}$ (no estimates of error given)	Salon *et al.* (1996a,b), Salon and Canvin (1997)
Estimate of CO_2 permeability of the carboxysome wall in *Anabaena variabilis* assuming all of the limitation to CO_2 efflux from carboxysomes is in the carboxysome wall. 30 °C	$2.8 \pm 0.8.10^{-7}$ (standard error, n=9)	McGinn *et al.* (1997)
Estimate of 'optimal' CO_2 permeability of cyanobacterial carboxysome wall from CCM model.	10^{-5}	Mangan and Brenner (2014)
CO_2 permeability of carboxysome wall in *Prochloroccus* estimated from a model of CCM function.	10^{-7}	Hopkinson *et al.* (2014)
Estimate of CO_2 permeability of the carboxysome wall in *Prochlorococcus* assuming all of the limitation to CO_2 efflux from carboxysomes is in the carboxysome wall.	10^{-6}	Hopkinson *et al.* (2014)

Method for all three data sets involves measurement of inorganic carbon fluxes, expressed as CO_2, under a known CO_2 concentration difference across the membrane across planar membrane bilayers (Gutknecht *et al.*, 1997; Missner *et al.*, 2008) or plasmalemma vesicles of *Chlamydomonas* (Sültemeyer and Rinast, 1996). Carbonic anhydrase was added to both sides of the membrane to minimize the gradient of CO_2 across the aqueous diffusion boundary layers on each side of the membrane.

Evans *et al.*, 2009; Niinemets *et al.*, 2009; Tazoe *et al.*, 2009, 2011). There is very significant interspecific variation in the magnitude of the mesophyll conductance (= permeability) of C_3 seed plants (Evans *et al.*, 2009; Niinemets *et al.*, 2009; Tazoe *et al.*, 2009; see Table 2).

Turning to submerged aquatic organisms, a number have CO_2 entry followed by diffusive flux to Rubisco, resembling C_3 land plants, although aquatic vascular plants lack stomata. These organisms include a number of freshwater and marine algae, aquatic bryophytes, and freshwater vascular plants (Raven, 1970; MacFarlane and Raven, 1985, 1989, 1990; Kübler *et al.*, 1999; Sherlock and Raven, 2001; Maberly and Madsen, 2002; Raven *et al.*, 2005; Maberly *et al.*, 2009; Maberly, 2014). While these organisms share some of the physiological characteristics found in organisms with CCMs, e.g. the absence of a competitive interaction between CO_2 and O_2 in photosynthetic gas exchange (Kübler *et al.*, 1999; Sherlock and Raven, 2001; Maberly *et al.*, 2009), the overall influence of environmental factors points to diffusive CO_2 entry.

CO_2 entry in organisms expressing a biophysical CCM

A biophysical CCM that involves diffusive CO_2 entry was first proposed by Walker *et al.* (1980; see Briggs, 1959) for ecorticate giant internodal cells of freshwater green algal macrophytes of the Characeae growing in relatively alkaline waters. The localized active efflux of H^+ across the plasmalemma causes a localized decrease in pH in the cell wall and diffusion boundary layer, to approximately 2 pH units below that in the medium. As HCO_3^- diffuses into the acid zone, the equilibrium CO_2:HCO_3^- increases 100-fold, as does the rate of HCO_3^- conversion to CO_2 in the absence of carbonic anhydrase (Walker *et al.*, 1980). Subsequently, expression of carbonic anhydrase in the acid zones was demonstrated, further increasing the rate of HCO_3^- to CO_2 conversion (Price *et al.*, 1985; Price and Badger, 1985). Intracellular acid-base regulation requires alkaline zones between the acid zones. This mechanism also occurs in some freshwater flowering plants where the acid zone is on the abaxial leaf surface and the alkaline zone is on the adaxial leaf surface (Maberly and Madsen, 2002). The high CO_2 concentration generated in the acid zones can, after crossing the plasmalemma by diffusion, give an internal CO_2 concentration rather less than that in the acid zone but still sufficient to constitute a CCM with the CO_2 concentration inside the cell higher than that in the bulk medium (Walker *et al.*, 1980; Price *et al.*, 1985; Price and Badger, 1985). As well as CO_2 leakage from the acid zones to the bulk medium, CO_2 could also leak from the cytosol back to the bulk medium through the alkaline zones.

A similar mechanism is thought to occur in many marine macrophytes as a mechanism of using external HCO_3^- (Raven and Hurd, 2012). However, the evidence for this is (a) inhibition of external HCO_3^- use by pH buffers that, ex hypothesis, eliminate the acid zones, (b) inhibition of external carbonic anhydrase using a membrane-impermeant inhibitor as well as, in some cases, (c) showing that photosynthesis is not decreased by inhibitors of one group of plasmalemma HCO_3^- transporters (Raven and Hurd, 2012). There have been no direct demonstrations of the acid zones in macroalgae because, although they can be visualized when they occur in the freshwater Characeae and vascular macrophytes, they must (if they exist!) occupy smaller areas in marine macroalgae and in seagrasses (Raven and Hurd, 2012).

An analogous mechanism involves external HCO_3^- entry at the plasmalemma and across the chloroplast envelope membranes, not necessarily giving higher internal than external concentration, with HCO_3^- entry to the thylakoid lumen via (ex hypothesis) HCO_3^--transporting channels (Raven, 1997b; Jungnick *et al.*, 2014; Raven *et al.*, 2014). The low pH of the thylakoid lumen, with the presence (at least in

Table 2. *Permeability coefficient ('mesophyll conductance') for CO_2 entry for the pathway from the outside of the external aqueous diffusion boundary layer to Rubisco in C_3 biochemistry*

No data seem to be available for the corresponding CO_2 movement to PEPc in C_4 or CAM biochemistry.

Category of plant: flowering plant, hornwort, or liverwort	Mesophyll permeability m s^{-1}
Herbaceous dicotyledonous flowering plant	$2.16 \pm 0.32.10^{-4}$ (standard error, *n* not clear)
Herbaceous monocotyledonous flowering plant	$2.24 \pm 0.29.10^{-4}$ (standard error, *n* not clear)
Woody deciduous dicotyledonous flowering plant	$1.05 \pm 0.12.10^{-4}$ (standard error, *n* not clear)
Woody evergreen dicotyledonous flowering plant	$0.85 \pm 0.08.10^{-4}$ (standard error, *n* not clear)
Hornwort	$1.75.10^{-4}$ (no statistics provided by Meyer *et al.*, 2008)
Unventilated liverwort	$1.90 \pm 0.15.10^{-4}$ (standard deviation, *n*=3
Ventilated liverwort	$0.80 \pm 0.04.10^{-4}$ (standard deviation, *n*=3)

Conversion of photosynthetic rates for the plants on a projected leaf area basis (from Table 1 of Warren, 2008) to the area of mesophyll cells exposed to the intercellular gas space uses a ratio of 25 m^2 m^2 mesophyll cells exposed to the intercellular gas space projected leaf area (from pp. 380–381of Nobel, 2005). Conversion of the difference in CO_2 concentration between the outside of the cell wall to the chloroplast stroma expressed in terms of atmospheric mol fraction (μmol CO_2 mol^{-1} total atmospheric gas) from Table 1 of Warren (2008) to mmol CO_2 dissolved in each m^3 of leaf water uses a conversion factor of 1 mmol CO_2 m^{-3} dissolved in leaf water for each 20.4 μmol CO_2 mol^{-1} total atmospheric gas (from pp. 377 and 384 of Nobel, 2005). For a ventilated thalloid liverwort the ratio of 9 m^2 mesophyll cells exposed to the intercellular gas space per m^2 projected thallus area (Green and Snelgar, 1982), and for a hornwort or and unventilated liverwort thallus the ratio is 1 (Green and Snelgar, 1982), with other data from Meyer *et al.* (2008).

Chlamydomonas) of a carbonic anhydrase, gives a rate of CO_2 production, and an equilibrium CO_2 concentration, similar to that in the extracellular acid zones of some freshwater macrophytes (Raven, 1997b; Moroney and Ynalvez, 2007; Jungnick *et al.*, 2014; Raven *et al.*, 2014). The final step in the CCM is diffusion of the CO_2 from the lumen to the stroma, and especially to the pyrenoid where most of the Rubisco occurs in *Chlamydomonas* (Raven, 1997b; Raven *et al.*, 2014). CO_2 leakage could occur from the pyrenoid back to the bulk medium.

Energetically downhill entry of CO_2 as part of a CCM occurs in cyanobacteria, although without localized surface acidification (see data of Maeda *et al.*, 2002, and models of Mangan and Brenner, 2014; Eichner *et al.*, 2015) (Fig. 1). Three further essential components are, first, active HCO_3^- influx at the plasmalemma, and the unidirectional conversion of CO_2 to HCO_3^- energized by the $NDHI_4$ component of cyclic electron flow round photosystem I at the outer surface of the thylakoid membrane. Second, the carboxysomes, containing Rubisco and carbonic anhydrase, whose protein subunit walls probably have a limited permeability to CO_2 (see estimates in Table 1). The cytosolic HCO_3^- from these two sources enters carboxysomes through pores also allowing permeation of anions (Raven, 2006) and H^+ (Menon *et al.*, 2010) or, perhaps, OH^-. Finally, HCO_3^- in the carboxysome lumen is acted on by carbonic anhydrase, producing CO_2 that is (mainly) consumed by Rubisco in the carboxysome, though some CO_2 could leak to the cytosol. The extent of CO_2 leakage through the carboxysomal wall is likely to be significant, even with a low CO_2 permeability coefficient across the carboxysomal wall with its positively charged pores, because of the large CO_2 accumulation factor (two to three orders of magnitude) in the carboxysome lumen relative to the cytosol during photosynthesis (Eichner *et al.*, 2015). Modelling by Mangan and Brenner (2014) finds that the optimal carboxysome wall permeability coefficient for CO_2 for maximal CO_2 accumulation in the carboxysome lumen is around 10^{-5} m s^{-1}.

Hopkinson *et al.* (2011) and Hopkinson (2014) propose a general similar mechanism for diatoms, with active HCO_3^- influx at the plasmalemma (Nakajima *et al.*, 2013) and parallel non-energized CO_2 influx (Fig. 2). These models involve cytosolic carbonic anhydrase to convert the CO_2 to the equilibrium concentration of HCO_3^-, with active HCO_3^- uptake by chloroplasts. This latter step has not yet been identified in diatoms.

Is there a role for active transport of CO_2? The occurrence of a CO_2-stimulated ATPase from the 'microsomal' fraction (= plasmalemma?) of the freshwater green (chlorophycean) alga *Eremosphaera viridis*, the predominance of CO_2 uptake in photosynthesis in this alga, and the electroneutrality of CO_2 uptake (ruling out cation symport), is consistent with CO_2 uptake by primary active transport (Rotatore *et al.*, 1992; Deveau *et al.*, 1998; Huertas *et al.*, 2000a; Deveau *et al.*, 2001). No other CO_2 transporters that could reasonably function in active CO_2 transport are known. Accordingly, the possibility that other eukaryotes depend on a mechanism of the kind suggested by Beardall (1981) and Hopkinson *et al.* (2011), involving passive CO_2 entry at the plasmalemma and active HCO_3^- transport into the chloroplasts, cannot be ruled out for algae with a CCM and dominant CO_2 uptake, e.g. acidophilic eukaryotic algae, unless it has been shown that there is no HCO_3^- transporter at the chloroplast envelope. Rotatore and Colman (1990) showed that isolated chloroplasts of *Chlorella ellipsoidea* could take up HCO_3^- by active

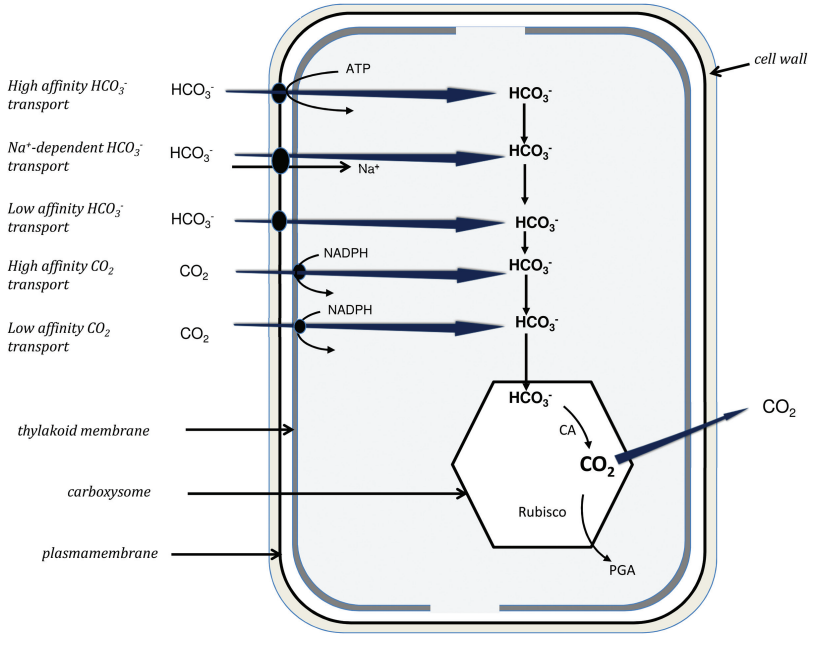

Fig. 1. A schematic model for inorganic carbon transport, and CO_2 accumulation and leakage in cyanobacteria. Low affinity transport systems are shown in grey and high affinity systems are shown in black, and are found at the plasmalemma and/or thylakoid membrane. Transporters whose characteristics are unknown are shown in white. Redrawn after Fig. 1 of Price *et al.* (2002), Badger and Price (2003), and Giordano *et al.* (2005). (Price *et al.* 2002. Modes of active inorganic carbon uptake in the cyanobacterium Synechocystis sp. PCC7942. Functional Plant Biology 29, 131–149. CSIRO PUBLISHING (http://www.publish.csiro.au/nid/102/paper/PP01229.htm). (Badger and Price 2003. CO_2 concentrating mechanism in cyanobacteria: molecular components, their diversity and evolution. Journal of Experimental Botany 54, 609–622). (Giordano *et al.* 2005. CO_2 concentrating mechanisms in algae: mechanisms, environmental modulation, and evolution. Annual Review of Plant Biology 6, 99–131).

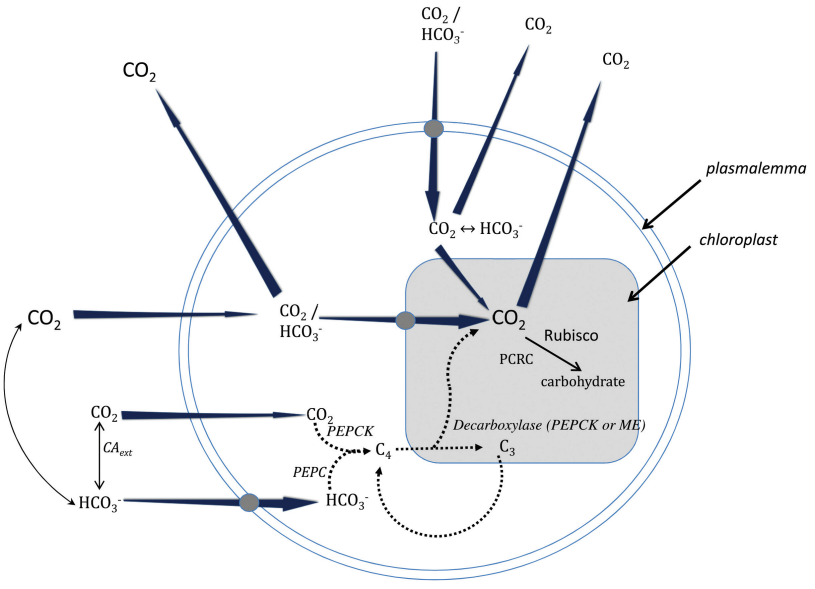

Fig. 2. A schematic model for inorganic carbon transport, and CO_2 accumulation and leakage in eukaryotic algal cells. The model incorporates the possibilities for DIC transport at the plasmalemma and/or chloroplast envelope as well as a putative C_4-like mechanism. Active transport processes (shown by the shaded boxes) can be of CO_2 or HCO_3^-. No attempt has been made to show the roles of the various internal CAs in the different compartments. For this the reader is referred to Giordano *et al.* (2005). Redrawn after Giordano *et al.* 2005. CO_2 concentrating mechanisms in algae: mechanisms, environmental modulation, and evolution. Annual Review of Plant Biology 6, 99–131.

transport, but had no active CO_2 uptake; however, the HCO_3^- influx uptake by isolated chloroplasts is less than that at the plasmalemma on a per cell basis (Rotatore and Colman, 1991a,b). Rotatore and Colman (1991c) suggest that there is active uptake of CO_2 at the plasmalemma of *Chlorella saccharophila* and *C. ellipsoidea*, although the possibility suggested by Beardall (1981) and Hopkinson *et al.* (2011) of passive CO_2 followed by active entry of inorganic C into chloroplasts cannot be ruled out.

HCO_3^- entry in organisms expressing a biophysical CCM

Use of HCO_3^- is indicated by more rapid photosynthesis than can be accounted for by the uncatalysed rate of HCO_3^- to CO_2 conversion (Briggs, 1959). The 'direct' use of HCO_3^- involves influx of the anion at the plasmalemma, as compared with the 'indirect' use by external conversion to CO_2 as described in the previous section (see Briggs, 1959). The physiological methods of demonstrating direct use of HCO_3^- involve the known absence, or inhibition, of external carbonic anhydrase(s). In cyanobacteria (Eichner *et al.*, 2015; Hopkinson *et al.*, 2014) and eukaryotic algae such as diatoms (Nakajima *et al.*, 2013), HCO_3^- entry has been shown by physiological methods, and also by molecular genetic techniques, including ectopic expression and tests of functionality of the HCO_3^- transporter gene. The processes in cyanobacteria (Mangan and Brenner, 2014; Eichner *et al.*, 2015) (Fig. 1) and diatoms (Hopkinson *et al.*, 2011; Hopkinson, 2014) (see Fig. 2) have been recently modelled.

In other algae HCO_3^- entry has been shown by physiological methods, including the absence of inhibition of photosynthesis by pH buffers or by inhibition of external carbonic anhydrase, and inhibition by inhibitors of anion exchange proteins (Raven and Hurd, 2012). In some cases, e.g. the eustigmatophycean marine microalga *Nannochloropsis gaditana*, all of the inorganic carbon entering in the CCM involves direct entry of HCO_3^- (e.g. Munoz and Merrett, 1989; Huertas and Lubián, 1997; Huertas *et al.*, 2000b, 2002). In most cases, there is entry of both CO_2 and HCO_3^- in CCMs (Korb *et al.*, 1997; Tortell *et al.*, 1997; Burkhardt *et al.*, 2001; Giordano *et al.*, 2005; Rost *et al.*, 2006a,b, 2007; Tortell *et al.*, 2008) and, in a few cases (see above) only CO_2 enters in algae with CCMs.

Isolated, metabolically active chloroplasts of some green algae with CCMs show CO_2 and HCO_3^- uptake into the chloroplasts as well as into whole cells (Amoroso *et al.*, 1998, van Hunnik *et al.*, 2002, Giordano *et al.*, 2005, and references therein). Yamano *et al.* (2015) show co-operative expression of the plasmalemma HCO_3^- HLA3 ABC transporter and the chloroplast envelope LCIA formate/nitrite transporter homologue (Wang and Spalding, 2014) in *C. reinhardtii.* LCIA is probably a HCO_3^- channel (Wang and Spalding, 2014; Yamano *et al.* 2015); Wang and Spalding (2014) point out that such a channel could not act to accumulate HCO_3^- in the stroma relative to the cytosol since the electrical potential difference across the chloroplast envelope is stroma negative relative to the cytosol.

Leakage from the intracellular inorganic C pool of CCMs

Significant attention has been paid to leakage of CO_2 from terrestrial C_4 plants; this has been thoroughly reviewed by Kromdijk *et al.* 2014 (Table 3 and Supplementary Table S1, available at *JXB* online). For typical C_4 anatomy with mesophyll cells with intercellular gas spaces and a single bundle sheath layer with limited exposure to intercellular gas spaces

and, in some cases, a suberin (mestome) sheath that could further limit CO_2 leakage, Kromdijk *et al.* (2014) give an excellent critique of the methods used to determine the leakiness to CO_2 (CO_2 efflux as a fraction of gross CO_2 influx) and list their outcomes. These are $^{14}CO_2$ labelling to determine the size of the bundle sheath inorganic carbon pool (and hence CO_2 efflux) or to directly estimate CO_2 efflux, the deviation of the quantum yield of CO_2 assimilation from the value predicted from biochemistry assuming no CO_2 leak, and the natural abundance of stable carbon isotopes in the organic matter of the plant (determined by destructive sampling, or from online measurements of CO_2 before and after gas flow over photosynthesizing plants) relative to that of source CO_2. All of these methods have problems (Kromdijk *et al.*, 2014; von Caemmerer *et al.*, 2014). The values for leakiness vary between –0.03–0.70. For the much less common case of terrestrial single-cell C_4 photosynthesis, the leakiness is similar to that for typical C_4 anatomy determined by similar methods (King *et al.*, 2012). The permeability of the bundle sheath cells for CO_2 ($1.6–4.5.10^{-6}$ m s^{-1}: Table 4), derived from the CO_2 efflux from the pool accumulated by the CCM and the driving force of the difference in CO_2 concentration between the CCM pool and the medium, is at least an order of magnitude higher than the permeabilities for cyanobacteria (Table 4). However, the bundle sheath permeability is two

Table 3. *Leakage of inorganic C from CCMs as a fraction of the inorganic C pumped into the intracellular pool for in terrestrial C_4 flowering plants, hornworts, eukaryotic algae, and cyanobacteria*

Values are from Supplementary Table S1 except for C_4 terrestrial flowering plants where the more detailed data in Table 1 of Kromdijk *et al.* (2014) was used. For C_3 plants, leakage of CO_2 from photorespiration is <0.2 of gross CO_2 fixation (see text).

Organism	Range of CO_2 leakage estimates as a fraction of gross CO_2 entry, from Supplementary Table S1	Mean leakage from estimates in Supplementary Table S1 or (C_4 terrestrial flowering plants) the more detailed data in Table 1 of Kromdijk *et al.* (2014)
C_4 terrestrial flowering plants	–0.03–0.70	0.260 ± 0.108 (standard deviation, *n*=20)[1]
Hornworts with CCMs	0.170, 0.304, 0.31	0.263 ± 0.066 (standard deviation, *n*=3)[2]
Eukaryotic algae	0.01–0.80	0.36 ± 0.16 (standard deviation, *n*=14): using results from MIMS only.[3]
Cyanobacteria	0.09–0.78	0.407 ± 0.214 (standard deviation, *n*=5): using results from MIMS only.[3]

[1]Calculated from sum of means of ranges in Table 1 of Kromdijk *et al.* (2014), using data from all methods of estimation. Where values are given for more than one irradiance the value from the highest irradiance was used. The theroretically impossible value of –0.03 of leakage obtained by the quantum yield methods was retained rather than being rounded to zero; this made no difference to the outcome.
[2]Estimates from C isotope method, acknowledging that the pyrenoid-based CCM in hornworts may be subject to over-estimation as a result of internal recycling discussed for eukaryotic algae (see Wang and Spalding, 2014).
[3]Estimates from the C isotope method for leakage from a cyanobacterium in excess of 1.0 are theoretically impossible; these and other very high values obtained by this method for the cyanobacteria, are not given here. Possible reasons for these very high values are discussed by Eichner *et al.* (2015). For eukaryotic algae an analogous over-estimate of leakage using the C isotope method to that suggested for cyanobacteria could also occur, at least in *Chlamydomonas* (Wang and Spalding, 2014), but in the case of the eukaryotic algae none of the leakage estimates from using the C isotope method in Supplementary Table S1 are higher than the highest estimates from the MIMS method.

Table 4. *Permeability coefficients, on a cell surface area basis, for CO_2 and HCO_3^- determined for efflux of inorganic carbon from the intracellular pool accumulated by CCMs in cyanobacteria and for the bundle sheath of C_4 plants*

Organism	Inorganic carbon species	Permeability coefficient m s^{-1}	Reference
Synechococcus (Cyanobacterium)	CO_2	10^{-7} m s^{-1} (no estimates of errors given)	Badger *et al.* (1985)
Synechococcus	CO_2	$2.49 \pm 0.13.10^{-8}$ m s^{-1} (standard error, n=4) $-3.36 \pm 0.14.10^{-8}$ m s^{-1} (standard error, *n*=18)	Salon *et al.* (1996a,b)
Synecchococcus[1]	HCO_3^-	$1.47 \pm 0.23.10^{-9}$ m s^{-1} (standard error, n = 7) $-1.84 \pm 0.17.10^{-9}$ m s^{-1} (standard error, *n*=7)	Salon *et al.* (1996a,b); Salon and Canvin (1997)
Anabaena variabilis (Cyanobacterium)	CO_2	$9.8 \pm 1.5.10^{-8}$ m s^{-1} (standard error, *n*=10)	McGinn *et al.* (1997)
Anabaena variabilis[1]	HCO_3^-	$7.6 \pm 0.9.10^{-9}$ m s^{-1} (standard error, *n*=7)	McGinn *et al.* (1997)
C_4 terrestrial flowering plants (5 species)	CO_2	$1.6–4.5.10^{-6}$ m s^{-1} (no estimates of errors given)	Furbank *et al.* (1989)

[1]The quantification of the efflux of HCO_3^- is less direct than that of CO_2 efflux. As mentioned by Salon *et al.* (1996b), the permeability coefficient for HCO_3^- is a minimal value since the inside-negative electrical potential difference across the plasmalemma is not accounted for in the calculations.

orders of magnitude less than the mesophyll permeability in C_3 plants (Table 2).

Leakage of an increased fraction of the CO_2 released into the bundle sheath by the biochemical CO_2 pump at low light is thought to be a reason for the rarity of shade-adapted C_4 plants (see Bellasio and Griffiths, 2014). Bellasio and Griffiths (2014) point out that there is an ontogenetic shading of older leaves in high light-adapted C_4 plants, and that up to 50% of C_4 crop photosynthesis occurs in shaded leaves, and investigated CO_2 leakage in shade-acclimated leaves of the sun-adapted *Zea mays*. They found that CO_2 leakage as a fraction of PEPc activity (= biochemical CO_2 pump) stayed constant with decreasing light, thus differing from expectation of a relative increase in leakage. The basis for the this constancy is a decreased PEPc activity relative to that of Rubisco, and fixation of an increased fraction of the CO_2 generated from respiration in bundle sheath cells.

Less attention has been paid to leakage of CO_2 from terrestrial CAM plants (Cockburn *et al.*, 1979; Winter and Smith, 1996; Nelson *et al.*, 2005; Nelson and Sage, 2008; Winter *et al.*, 2015).

Cockburn *et al.* (1985) examined the shootless orchid *Chiloschista usneoides* where CAM occurs (in the absence of other photosynthetic structures) in the astomatous velameniferous root. The absence of stomata means that the usual terrestrial CAM method of diurnal closure of stomata decreasing CO_2 leakage during deacidification and CO_2 refixation by Rubisco is unavailable. Cockburn *et al.* (1985) showed that the intercellular CO_2 concentration during deacidification is not significantly different from that of the surrounding atmosphere, while the intercellular CO_2 concentration during dark acidification is lower than that of the surrounding atmosphere. While lower intercellular CO_2 concentration in the deacidification phase than is the case of stomata-bearing CAM structures decreases the leakage of CO_2 from intercellular gas spaces in the stomata-less roots, it also means that carboxylase activity of Rubisco is likely to be substantially below saturation, and the Rubisco oxygenase activity is likely to be significant.

For aquatic vascular plants with CAM there is also no possibility of stomatal limitation of leakage of CO_2 produced during deacidification in the light. For isoetids there is very little loss from the possible leakage of CO_2 from the astomatal, cuticularized, photosynthetic part of the leaf, and even loss from any lower, less cuticularized, part of the leaf might be limited or abolished by the high CO_2 concentration in the surrounding sediment that contains mineralizing particulate organic matter derived by sedimentation from the plankton. The non-isoetid submerged aquatic CAM flowering plant *Crassula helmsii* also lacks the leakage-limiting stomatal closure mechanism of terrestrial *Crassula* spp. *C. helmsii* can show net CAM fixation from external CO_2 in the dark and also net photosynthetic C_3 CO_2 assimilation from external CO_2 in parallel with refixation of internal CO_2 generated in deacidification from malic acid, with, presumably, implications for CO_2 leakage (Newman and Raven, 1995; Maberly and Madsen, 2002; Klavsen and Maberly, 2010).

Of course, the great majority of aquatic primary producers carrying out almost all of the aquatic primary productivity involving CCMs do not express CAM. Essentially all of the work on leakage of CO_2 from intracellular pools of the CCM in aquatic organisms comes from cyanobacteria and eukaryotic microalgae; very little is known of leakage of CO_2 from algal macrophytes or submerged aquatic vascular macrophytes that concentrate CO_2 by C_4 metabolism or a biophysical CCM. Perhaps the clearest example of CO_2 leakage comes from the work of Tchernov *et al.* (1997, 1998, 2003) using membrane inlet mass spectrometry (MIMS). This method gives estimates of changes in CO_2 and O_2 in solution, with the difference between the two (if the photosynthetic quotient is assumed to be 1) representing the HCO_3^- flux, typically (see above) HCO_3^- influx. Tchernov *et al.* (1997, 1998, 2003) found an increase in external O_2 and also CO_2, with the computed HCO_3^- influx exceeding the organic carbon production rate computed from O_2 production. Especially at high light, the HCO_3^- influx can significantly exceed the rate of photosynthesis, with the 'excess' inorganic carbon lost as CO_2 in (for example) the cyanobacterium *Synechococcus* and the eustigmatophycean eukaryotic alga *Nannochloropsis* (Tchernov *et al.*, 1997, 1998, 2003). There are also cases of CO_2 influx exceeding the organic C production, implying net HCO_3^- efflux.

However, the general case with MIMS measurements is that of CO_2 decrease, or at least no increase, in the light. Here the MIMS method can be used to estimate CO_2 efflux in the light from the CO_2 efflux immediately after the cessation of illumination (Badger *et al.*, 1994; Salon *et al.*, 1996a,b; Eichner *et al.*, 2015; Table 3 and Supplementary Table S1, available at *JXB* online). Badger *et al.* (1994) found a leakage of not more than 0.1 of net photosynthesis in low inorganic carbon-grown cells of *Synechococcus*, while for low CO_2 grown *Chlamydomonas* the corresponding leakage is 0.5 at low inorganic C and 0.1 at high inorganic C. Again using *Synechococcus*, Salon *et al.* (1996a,b) and Salon and Canvin (1997) were able to distinguish CO_2 efflux from HCO_3^- efflux immediately after darkening; the total inorganic C efflux in the presence of carbonic anhydrase was measured, as was the CO_2 efflux under non-equilibrium conditions, and the difference is the HCO_3^- efflux. The CO_2 efflux was only 0.08 of the maximum CO_2 influx, while the HCO_3^- efflux was 0.45 of the maximum HCO_3^- influx. The CO_2 permeability coefficient determined from the measurements and expressed in terms of the plasmalemma area was 3.10^{-8} m s–^{1}, while it was 1.6–2.5 m s^{-1} in terms of the carboxysome area (Tables 1, 14). The HCO_3^- permeability coefficient expressed in terms of the plasmalemma area is at most 1.4–$1.7.10^{-9}$ m s^{-1} (Table 4); the value is an upper limit because the inside-negative electrical potential component was not used in the calculation (Salon *et al.* 1996b; see also Ritchie *et al.* 1996).

In the case of *Trichodesmium* the leakage (CO_2 efflux:gross inorganic carbon uptake) calculated using MIMS is 0.3–0.7 for two CO_2 levels and with or without NO_3^- (Eichner *et al.*,

2015), as compared with values of 0.5–0.9 in previous work on this organism (see Kranz *et al.*, 2009, 2010) (Table 3).

The other main method for estimating leakage of CO_2 from aquatic organisms expressing a CCM is from natural abundance $^{13}C/^{12}C$ of particulate organic matter gained by photolithotrophic growth and of the $^{13}C/^{12}C$ of external inorganic carbon species (Sharkey and Berry, 1985; Eichner *et al.*, 2015; Table 3 and Supplementary Table S1). This method is also used for estimating leakage of CO_2 from terrestrial C_4 plants (see above). Eichner *et al.* (2015) found a difference between the MIMS and the natural abundance $^{13}C/^{12}C$ estimates of leakage, with the latter method giving values of the 0.82 and 1.14. They point out that the values > 1 are theoretically impossible; Eichner *et al.* (2015) suggest kinetic fractionation between CO_2 and HCO_3^- in the cytosol and/or enzymatic fraction by the 'energized, unidirectional carbonic anhydrase' NDH-1_4 as possible causes of the very high leakage estimates. An analogous role might be played by the LCIA/LCIB system in *C. reinhardtii* (Wang and Spalding, 2014), so that estimates of leakage from carbon isotope ratios may be too high in *Chlamydomonas* and possibly in other eukaryotic algae as well. This possibility is acknowledged in Table 3 and Supplementary Table S1 (available at *JXB* online).

The mean value for the leakage determined by MIMS for cyanobacteria and eukaryotic algae in Supplementary Table S1 is, as indicated in Table 3, respectively 0.407 ± 0.214 (standard deviation, n=5) and 0.36 ± 0.16 (standard deviation, n=14). The mean values for hornworts with CCMs and C_4 terrestrial flowering plants are 0.263 ± 066 (standard deviation, n=3) and 0.260 ± 0.106 (standard deviation, n=20), respectively. There is a trend (not significant) for lower fractional leakage in terrestrial C_4 plants and hornworts than for cyanobacteria and eukaryotic algae.

As for C_4 plants, so with cyanobacterial and algal CCMs: the prediction is an increasing fraction of the inorganic C pumped into the intracellular pool being lost as CO_2 efflux with decreasing incident photosynthetically active radiation, and that algae relying on diffusive CO_2 entry from the medium to Rubisco would be more common in low-irradiance habitats (review by Raven *et al.* 2000). The limited data available agree with these predictions (Raven *et al.*, 2000, 2002; Burkhardt *et al.*, 2001; de Araujo *et al.*, 2011; Cornwall *et al.*, 2015; see Table 3). Turning to temperature, Raven and Beardall (2014) show that algal CCMs occur at lower temperatures than does terrestrial C_4 photosynthesis. Kranz *et al.* (2015) showed that the energy cost of algal CCMs decreased at low temperatures; it is not known if this is the case for terrestrial C_4 photosynthesis.

Leakage from the photorespiratory carbon oxidation cycle(s)

Tcherkez (2013) gives an excellent critique of the CO_2 fluxes associated with C_3 photosynthesis, photorespiration, and respiration. With a carboxylase:oxygenase ratio of Rubisco *in vivo* in a C_3 plant in the present atmosphere of 3:1, CO_2 production in the photorespiratory carbon oxidation cycle is 0.167 of gross CO_2 assimilation in photosynthesis (Raven, 1972a,b; Tcherkez, 2013). There is about 15% recycling of the photorespiratory CO_2 and 'dark' respiratory CO_2 production in photosynthesizing structures (Raven, 1972a,b; Tcherkez, 2013), so the CO_2 release into the environment as a fraction of gross photosynthesis is 0.167×0.85 or 0.14; it is likely that an upper limit is 0.20. This is at the low end of the range for leakage in C_4 plants and in algae CCMs (Table 3).

The various C_3–C_4 intermediate flowering plants have photosynthetic gas exchanges that show varying mixtures of C_3 and C_4 characteristics (Hylton *et al.*, 1988; Rawsthorne *et al.*, 1988a,b; von Caemmerer, 1989; Rawsthorne and Hylton, 1991; Morgan *et al.*, 1993). This work shows the expression of most or all the glycine decarboxylase activity, and some of the Rubisco carboxylase–oxygenase, in bundle sheath cells. This location of the decarboxylase of the photorespiratory carbon oxidation cycle, with some Rubisco, in tightly packed bundle sheath cells increases recycling of CO_2 from glycine decarboxylase by the carboxylase activity of Rubisco relative to leakage of CO_2 back to the intercellular spaces.

CCMs increase the steady-state CO_2:O_2 ratios at the site of Rubisco activity; this decreases the ratio of Rubisco oxygenase activity to that of Rubisco carboxylase activity. The decreased rate of production of phosphoglycolate involves a decreased rate of the pathway(s) converting phosphoglycolate into phosphoglycerate and triose phosphate that can be used in core metabolism and/or complete oxidation to CO_2 (Eisenhut *et al.*, 2008; Hagemann *et al.*, 2010; Young *et al.*, 2011; Raven *et al.*, 2012). Even this low flux is essential, since deletion of all three of the pathways of phosphoglycolate metabolism (photorespiratory carbon oxidation cycle; tartronic semialdehyde pathway; complete oxidation via oxalate) is lethal (Eisenhut *et al.*, 2008; Hagemann *et al.*, 2010; Raven *et al.*, 2012). Comparable work has not been yet been carried out in photosynthetic eukaryotes with CCMs where, at least in embryophytes, the pathway of phosphoglycolate metabolism is the photorespiratory carbon oxidation cycle. However, it is known that the C_4 and CAM CCMs decrease the rate of phosphoglycolate synthesis and flux through the photorespiratory carbon oxidation cycle relative to what occurs in otherwise comparable C_3 plants.

Conclusions

Quantifying the flux of CO_2 into and out of cells is difficult. All known irreversible decarboxylases produce CO_2; CO_2 is also the product/substrate of enzymes that can act as carboxylases and decarboxylases. Whether reversible of irreversible, decarboxylases produce CO_2, which can potentially leak out of cells. Some irreversible carboxylases also have CO_2 as their substrate; others use HCO_3^-.

There is still controversy as to the relative role of permeation through the lipid bilayer and of movement through membrane proteins such as CO_2-selective aquaporins in the downhill, non-energized, movement of CO_2. Such movement is involved in CO_2 entry in terrestrial and aquatic organisms with C_3 physiology and biochemistry, as well as terrestrial C_4

plants and all CAM plants. Although there is also some evidence of active CO_2 transport at the plasmalemma of algae, downhill CO_2 transport is part of some mechanisms involved in the use of external HCO_3^- and CCM function. Further work is needed to test the validity of the mechanism based on localized surface acidification in marine macrophytes, and on HCO_3^- conversion to CO_2 in the thylakoid lumen.

HCO_3^- active influx at the plasmalemma underlies all cyanobacterial and some algal CCMs. HCO_3^- can also enter chloroplasts of some algae, possible as part of a CCM. Leakage from the intracellular CO_2 and HCO_3^- pool of CCMs sometimes occurs as HCO_3^-, but typically occurs as CO_2. Leakage from cyanobacterial and microalgal CCMs, and terrestrial C_4 plants and hornworts with CCMs, usually involve half or less of the gross inorganic C entering in the CCM, but can be as high as 80%. CO_2 leakage to the environment from photorespiration in C_3 plants is less than 20% of gross photosynthesis. Leakage from terrestrial CAM plants, algal macrophytes, and vascular aquatic macrophytes with CCMs (C_4, CAM, biophysical CCMs) has been less extensively examined. From what is known, CO_2 leakage can be appreciable in many photoautotrophs with CCMs and increases the energetic cost of net inorganic carbon fixation (see Raven *et al.*, 2014).

Supplementary data

Supplementary data are available at *JXB* online.

Table S1. Leakage of inorganic C from CCMs as a fraction of the inorganic C pumped into the intracellular pool.

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References

Amoroso G, Sültemeyer D, Thyssen C, Fock HP. 1998. Uptake of HCO_3^- and CO_2 in cells and chloroplasts from the microalgae *Chlamydomonas reinhardtii* and *Dunaliella tertiolecta*. Plant Physiology **116,** 193–201.

Badger MR, Bassett M, Comins HN. 1985. A model for HCO_3^- accumulation and photosynthesis in the cyanobacterium *Synechococcus* sp. Plant Physiology **77,** 465–471.

Badger MR, Palmqvist K, Yu JW. 1994. Measurements of CO_2 and HCO_3^- fluxes in cyanobacteria and microalgae during steady-state photosynthesis. Physiologia Plantarum **90,** 529–536.

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

Beardall J. 1981. CO_2 accumulation by *Chlorella saccharophila* (Chlorophyceae) at low external pH: evidence for active transport of inorganic carbon at the chloroplast envelope. Journal of Phycology **17,** 371–373.

Bellasio C, Griffiths H. 2014. Acclimation of low light by C4 maize: implications for bundle sheath leakiness. Plant Cell and Environment **37,** 1046–1058.

Boron WF, Endeward V, Gros G, Musa-Aziz R, Pohl P. 2011. Intrinsic CO_2 permeability of cell membranes and potential biological relevance of CO_2 channels. ChemPhysChem **12,** 1017–1019.

Briggs GE. 1959. Bicarbonate ions as a source of carbon dioxide for photosynthesis. Journal of Experimental Botany **10,** 90–92.

Burkhardt S, Amoroso G, Riebesell U, Sültemeyer D. 2001. CO_2 and HCO_3^- uptake in marine diatoms acclimated to different CO_2 concentrations. Limnology and Oceanography **46,** 1378–1391.

Cockburn W, Ting IP, Sternberg LO. 1979. Relationship between stomatal behaviour and internal carbon dioxide concentrations in Crassulacean Metabolism plants. Plant Physiology **63,** 1029–1032.

Cockburn W, Goh CJ, Avadhani, PN. 1985. Photosynthetic carbon assimilation in a shootless orchid, *Chiloschista usenoides* (DON) LDL. A variant on crassulacean acid metabolism. Plant Physiology **77,** 83–86.

Colman B, Espie GA. 1985. CO_2 uptake and transport in leaf mesophyll cells. Plant Cell and Environment **8,** 449–457.

Cornwall CE, Revill AT, Hurd CL. 2015. High prevalence of diffusive uptake by CO_2 by macroalgae in a temperate subtidal system. Photosynthesis Research **124,** 181–190.

de Araujo ED, Patel J, de Araujo C, Rogers SP, Short SM, Cambell DA, Espie GS. 2011. Physiological characterization and light response of the CO_2-concentrating mechanism in the filamentous cyanobacterium *Leptolyngbya* sp. CPPP 696. Photosynthesis Research **109,** 95–103.

Deveau JST, Khosravari H, Lew RR, Colman B. 1998. CO_2 uptake mechanism in *Eremosphaera viridis.* Canadian Journal of Botany **76,** 1161–1164.

Deveau JST, Lew RR, Colman B. 2001. Evidence for active CO_2 uptake by a CO_2-ATPase in the acidophilic green alga *Eremosphaera viridis.* Canadian Journal of Botany **79,** 1274–1281.

Eichner M, Thoms S, Kranz SA, Rost B. 2015. Cellular inorganic carbon fluxes in *Trichodesmium*: a combined approach using measurements and modelling. Journal of Experimental Botany **66,** 749–759.

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

Endeward V, Al-Samir S, Itel F, Gros G. 2014. How does carbon dioxide permeate cell membranes? A discussion of concepts, results and methods. Frontiers in Physiology **4**, Article 382, pp.1–21.

Espie GS, Colman B. 1986. Inorganic carbon uptake during photosynthesis. I. A theoretical analysis using the isotope disequilibrium technique. Plant Physiology **80,** 863–869.

Espie GS, Owttrim GW, Colman B. 1986. Inorganic carbon uptake during photosynthesis. II. Uptake by isolated *Asparagus* mesophyll cells during isotope disequilibrium. Plant Physiology **80,** 870–876.

Evans JR, Kaldenhoff R, Genty B, Terashima I. 2009. Resistances along the CO_2 diffusion pathway inside leaves. Journal of Experimental Botany **60,** 2235–2248.

Field CB, Behrenfeld J, Randerson PG, Falkowski PG. 1998. Primary production in the biosphere: integrating terrestrial and oceanic components. Science **29,** 737–740.

Firestyne SM, Wu W, Youn H, Davison VJ. 2009. Interrogating the mechanism of a tight-binding inhibitor of AIR carboxylase. Bioinorganic and Medicinal Chemistry **17,** 794–803.

Furbank RT, Jenkins CLD, Hatch MD. 1989. CO_2 concentrating mechanism of C_4 photosynthesis: permeability of isolated bundle sheath cells to inorganic carbon. Plant Physiology **91,** 1364–1371.

Giordano M, Beardall J, Raven JA. 2005. CO_2 concentrating mechanisms in algae: mechanisms, environmental modulation, and evolution. Annual Review of Plant Biology **6,** 99–131.

Green TGA, Snelgar WP. 1982. A comparison of photosynthesis in two thalloid liverworts. Oecologia **54,** 275–280.

Gutknecht J, Bisson MA, Tosteson DC. 1977. Diffusion of carbon dioxide through lipid bilayer membranes. Effects of carbonic anhydrase, bicarbonate and unstirred layers Journal of General Physiology **69,** 779–794.

Hagemann M, Eisenhut M, Hackenberg C, Bauwe H. 2010. Pathway and importance of photorespiratory 2-phosphoglycolate metabolism in cyanobacteria. Advances in Experimental Biology and Medicine **675,** 91–108.

Hanba YT, Shibasaka M, Hayashi Y, Hayakawa T, Kasamo K, Terashima I, Katsuhara M. 2004. Overexpression of the barley aquaporin HvPIP2;1 increases internal CO_2 conductance and CO_2 assimilation in the leaves of transgenic rice plants. Plant and Cell Physiology **45,** 521–529.

Häusler RE, Holtum JAM, Latzko E. 1987. CO_2 is the inorganic carbon substrate of $NADP^+$ malic enzymes from *Zea mays* and from wheat germ. European Journal of Biochemistry **163,** 619–626.

Heckwolf M, Pater D, Hanson DT, Kaldenhoff R. 2011. The *Arabidopsis thaliana* aquaporin AtPIP1;2 is a physiologically relevant CO_2 transport facilitator. The Plant Journal **67,** 795–804.

Hopkinson BM. 2014. A chloroplast pump model for the CO_2 concentrating mechanism in the diatom *Phaeodactylum tricornutum*, Photosynthesis Research **121,** 223–233.

Hopkinson BM, Dupont CL, Allen AE, Morel FMM. 2011. Efficiency of the CO_2-concentrating mechanism of diatoms. Proceedings of the National Academy of Sciences USA **108,** 3830–3837.

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

Huertas IE, Lubián LM. 1997. Comparative study of dissolved inorganic carbon and photosynthetic responses in *Nannochloris* (Chlorophyceae) and *Nannochloropsis* (Eustigmatophyceae) species. Canadian Journal of Botany **76,** 1104–1108.

Huertas IE, Colman B, Espie GS, Lubián LM. 2000a. Active transport of CO_2 by three species of marine microalgae, Journal of Phycology **36,** 314–320.

Huertas IE, Espie GS, Colman B, Lubián LM. 2000b. Light-dependent bicarbonate uptake and CO_2 efflux in the microalga *Nannochloropsis gaditana.* Plants **211,** 43–49.

Huertas IE, Lubián LM, Espie GS. 2002. Mitochondrial-driven bicarbonate transport supports photosynthesis in a marine microalga. Plant Physiology **130,** 284–291.

Hylton CM, Rawsthorne S, Smith AM, Jones DA, Woolhouse HW. 1988. Glycine decarboxylase is confined to the bundle-sheath cells of leaves of C_3-C_4 intermediate species of *Moricandia.* Planta **175,** 452–459.

Itel F, Al-Samir S, Öberg F, *et al*. 2012. CO_2 permeability of cell membranes is regulated by membrane cholesterol and protein gas channels. The FASEB Journal **26,** 5182–5191.

Jenkins CLD, Burnell JN, Hatch MD. 1987. Form of inorganic carbon involved as a product and as an inhibitor in C_4 photosynthesis. Plant Physiology **85,** 952–957.

Jungnick N, Ma Y, Mukherjee B, Cronan JC, Speed DJ, Laborde SM, Longstreth DJ, Moroney JV. 2014. The carbon concentrating mechanism in *Chlamydomonas reinhardtii*: finding the missing pieces. Photosynthesis Research **121,** 159–173.

Kai L, Kaldendorf R. 2014. A refined model of water and CO_2 membrane diffusion: Effects and contribution of sterols and proteins. Scientific Reports **4,** 6665. doi: 10.1028/srep06665.

King JL, Edwards GE, Cousins A. 2012. The efficiency of the CO_2-concentrating mechanism during single-cell C_4 photosynthesis. Plant Cell and Environment **33,** 1935–1948.

Klavsen SK, Maberly SC. 2010. The effect of light and CO_2 on inorganic carbon uptake in the invasive aquatic CAM-plant *Crassula helmsii.* Functional Plant Biology **37,** 727–747.

Korb RE, Saville PJ. Johnston AM, Raven JA. 1997. Sources of inorganic carbon for photosynthesis by three species of marine diatoms. Journal of Phycology **33,** 433–440.

Kranz SA, Sültemeyer D, Richter K-U, Rost B. 2009. Carbon acquisition in *Trichodesmium*: the effect of pCO_2 and diurnal changes. Limnology and Oceanography **54,** 548–559.

Kranz SA, Levitan O, Richter K-U, Prasil O, Berman-Frank I, Rost B. 2010. Combined effects of CO_2 and light on the N_2 fixing cyanobacterium *Trichodesmium* IMS101: physiological responses. Plant Physiology **154,** 334–345.

Kranz S, Young JN, Goldman J, Tortell PD, Bender M, Morel FMM. 2015. Low temperature reduces the energetic requirement for the CO_2 concentrating mechanism in diatoms. New Phytologist **205,** 192–201.

Kromdijk J, Ubierna N, Cousins AB, Griffiths H. 2014. Bundle-sheath leakiness in C_4 photosynthesis: a careful balancing act between CO_2 concentration and assimilation. Journal of Experimental Botany **65,** 3443–3457.

Kübler JE, Johnston AM, Raven JA. 1999. The effects of reduced and elevated CO_2 and O_2 on the seaweed, *Lomentaria articulata*. Plant, Cell and Environment **22,** 1303–1310.

Maberly SC. 2014. The fitness of the environments of air and water for photosynthesis, growth, reproduction and dispersal of photoautotrophs: an evolutionary and biogeochemical perspective. Aquatic Botany **118,** 4–13.

Maberly SC, Madsen TV, 2002. Freshwater angiosperm carbon concentrating mechanisms: processes and patterns. Functional Plant Biology **29,** 393–405.

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

MacFarlane JJ, Raven JA. 1985. External and internal CO_2 transport in *Lemanea:* interactions with the kinetics of ribulose bisphosphate carboxylase. Journal of Experimental Botany **36,** 610–622.

MacFarlane JJ, Raven JA. 1989. Quantitative determination of the unstirred layer permeability and kinetic parameters of RUBISCO in *Lemanea mamillosa*. Journal of Experimental Botany **40,** 321–327.

MacFarlane JJ, Raven JA. 1990. C, N and P nutrition of *Lemanea mamillosa* Kutz. (Batrachospermales, Rhodophyta) in the Dighty Burn, Angus, Scotland. Plant, Cell and Environment **13,** 1–13.

Maeda S-I, Badger MR, Price GD. 2002. Novel gene products associated with NdhD3/D4-containing NDH1 complexes are involved in photosynthetic CO_2 hydration in the cyanobacterium, *Synechocystis* sp. Molecular Microbiology **43,** 425–435.

Mangan NM, Brenner MP. 2014. Systems analysis of the CO_2 concentrating mechanism in cyanobacteria. eLife **3,** e2043. doi: 10.7554/eLife.02043. see also Correction published 29 April 2014.

McGinn PJ, Coleman JR, Canvin DT. 1997. Influx and efflux of inorganic carbon during steady-state photosynthesis of air-grown *Anabaena variabilis.* Canadian Journal of Botany **75,** 1913–1926.

Menon BB, Heihorst S, Shively JM, Canon GC. 2010. The carboxysome shell is permeable to protons. Journal of Bacteriology **192,** 5881–5886.

Meyer M, Seibt U, Griffiths H. 2008. To concentrate or ventilate. Carbon acquisition, isotope discrimination and physiological ecology of early land plant life forms. Philosophical Transactions of the Royal Society of London B **363,** 2767–2778.

Missner A, Kügler P, Saparov SM, Sommer K, Mathai JC, Zeidel ML. 2008. Carbon dioxide transport through membranes. Journal of Biological Chemistry **282,** 25340–25347.

Morgan CL, Turner SR, Rawsthorne S. 1993. Coordination of the cell-specific of the four subunits of glycine decarboxylase and of serine hydroxymethyltrasnferase in leaves of C_3-C_4 intermediate species from different genera. Planta **190,** 468–473.

Moroney JV, Ynalvez RA. 2007. Proposed carbon dioxide concentrating mechanism in *Chlamydomonas reinhardtii.* Eukaryotic Cell **6,** 1251–1259.

Munoz J, Merrett MJ. 1989. Inorganic-carbon transport in some marine eukaryotic microalgae. Planta **178,** 450–455.

Nakajima K, Tanaka A, Matsuda Y. 2013. SLC4 family transporters in a marine diatom directly pump bicarbonate from seawater. Proceedings of the National Academy of Sciences USA **110,** 1767–1772.

Nelson EA, Sage TL, Sage RF. 2005. Functional leaf anatomy of plants with crassulacean acid metabolism. Functional Plant Biology **32,** 409–419.

Nelson EA, Sage RF. 2008. Functional constraints of CAM leaf anatomy: tight cell packing is associated with increased CAM function across a gradient of CAM expression. Journal of Experimental Botany **59,** 1841–1850.

Newman JR, Raven JA. 1995. Photosynthetic carbon assimilation by *Crassula helmsii.* Oecologia **101,** 494–499.

Niinemets Ü, Wright IJ, Evans JR. 2009. Leaf mesophyll conductance in 35 Australian sclerophylls covering a broad range of foliage structure and physiological variation. Journal of Experimental Botany **60,** 2433–2449.

Nobel PS. 2005. Physicochemical and environmental plant physiology. 3rd ed Amsterdam: Elsevier.

Price GD, Badger MR. 1985. Inhibition by proton buffers of photosynthetic utilization of bicarbonate in *Chara corallina.* Australian Journal of Plant Physiology **12,** 257–267.

Price GD, Badger MR, Bassett ME, Whitecross MI. 1985. Involvement of plasmalemmasomes and carbonic anhydrase in photosynthetic utilization of bicarbonate in *Chara corallina.* Australian Journal of Plant Physiology **12,** 241–256.

Price GD, Maeda S-I, Omata T, Badger MR. 2002. Modes of active inorganic carbon uptake in the cyanobacterium *Synechocystis* sp. PCC7942. Functional Plant Biology **29,** 131–149.

Raven JA. 1970. Exogenous inorganic carbon sources in plant photosynthesis. Biology Reviews **45,** 167–221.

Raven JA. 1972a. Endogenous inorganic carbon sources in plant photosynthesis. I. Occurrence of the dark respiratory pathways in illuminated green cells. New Phytologist **71,** 227–247.

Raven JA. 1972b. Endogenous inorganic carbon sources in plant photosynthesis. II. Comparison of total CO_2 production in the light with measured CO_2 evolution in the light. New Phytologist **71,** 995–1014.

Raven JA. 1984. Energetics and Transport on Aquatic Plants. A R Liss, New York, NY. pp. ix + 587.

Raven JA. 1997a. Inorganic acquisition by marine autotrophs. Advances in Botanical Research **27,** 85–209.

Raven JA. 1997b. CO_2 concentrating mechanisms: a direct role for thylakoid lumen acidification? Plant, Cell and Environment, 147–154.

Raven JA. 2006. Sensing inorganic carbon: CO_2 and HCO_3^-. Biochemical Journal **396,** e5–e7.

Raven JA, Glidewell SM. 1981. Processes limiting photosynthetic conductance. In: Physiological Processes Limiting Plant Productivity (ed. by C.B. Johnson), 109–136. Butterworths, London.

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

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

Raven JA, Ball LA, Beardall J, Giordano M, Maberly SC. 2005. Algae lacking carbon concentrating mechanisms. Canadian Journal of Botany **83,** 879–890.

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 B **367,** 493–507.

Raven JA, Hurd CJ. 2012. Ecophysiology of photosynthesis in macroalgae. Photosynthesis Research **113,** 105–125.

Raven JA, Beardall J. 2014. CO_2 concentrating mechanisms and environmental change. Aquatic Botany **118,** 24–37.

Raven JA, Beardall J, Giordano M. 2014. Energy costs of carbon dioxide concentrating mechanisms in aquatic organisms. Photosynthesis Research **121,** 111–124.

Rawsthorne S, Hylton CM, Smith AM, Woolhouse HW. 1988a. Photorespiratory metabolism and immunogold localization of photorespiratory enzymes in C_3 and C_3-C_4 intermediate species of *Moricandia.* Planta **173,** 298–308.

Rawsthorne S, Hylton CM, Smith AM, Woolhouse HW. 1988b. Photorespiratory metabolism and immunogold localization of photorespiratory enzymes in C_3 and C_3-C_4 intermediate species of *Moricandia.* Planta **176,** 527–532.

Rawsthorne S, Hylton CM. 1991. The relationship between the post-illumination CO_2 burst and glycine metabolism in leaves of C_3 and C_3-C_4 intermediate species of *Moricandia.* Planta **186,** 122–126.

Reinhold L, Zviman M, Kaplan A. 1987. Inorganic carbon fluxes in cyanobacteria: a quantitative model. In: J Biggins, ed. Progress in Photosynthesis. Martinus Nijhof, Dordrecht, The Netherlands, pp. 289–296.

Reinhold L, Kosloff R, Kaplan A. 1991. A model for inorganic carbon fluxes and cyanobacterial carboxysomes. Canadian Journal of Botany **69,** 984–988.

Ritchie RJ, Nadolny C, Larkum AWD. 1996. Driving forces for bicarbonate transport in the cyanobacterium *Synechococcus R-2* (PCC 7942). Plant Physiology **112,** 1573–1584.

Rost B, Riebesell U, Sültemeyer D. 2006a. Carbon acquisition of marine phytoplankton. Effect of photoperiod length. Limnology and Oceanography **51,** 12–20.

Rost B, Richter K-U, Riebesell U, Hansen PJ. 2006b. Inorganic carbon acquisition by red tide dinoflagellates. Plant Cell and Environment **29,** 810–822.

Rost B, Kranz SA, Richter K-U, Tortell PD. 2007. Isotope disequilibrium and mass spectrometric studies of inorganic carbon acquisition by phytoplankton. Limnology and Oceanography: Methods **5,** 328–337.

Rotatore C, Colman B. 1990. Uptake of inorganic carbon by isolated chloroplasts of *Chlorella ellipsoidea.* Plant Physiology **93,** 1597–1600.

Rotatore C, Colman B. 1991a. The localization of active inorganic carbon transport at the plasma membrane in *Chlorella ellipsoidea.* Canadian Journal of Botany **69,** 1025–1031.

Rotatore C, Colman B. 1991b. The active uptake of carbon dioxide by the unicellular green algae transport *Chlorella saccharophila* and *C. ellipsoidea.* Plant Cell and Environment **14,** 371–375.

Rotatore C, Colman B. 1991c. The acquisition and accumulation of inorganic carbon by the unicellular green algae transport *Chlorella ellipsoidea.* Plant Cell and Environment **14,** 377–382.

Rotatore C, Lew RR, Colman B. 1992. Active uptake of CO_2 during photosynthesis in the green alga *Eremosphaera viridis* is mediated by a CO_2-ATPase. Planta **188,** 539–545.

Salon C, Mir NA, Canvin DT. 1996a. Influx and efflux of inorganic carbon in *Synechococcus* UTEX 625. Plant Cell and Environment **19,** 247–259.

Salon C, Mir NA, Canvin DT. 1996b. HCO_3^- and CO_2 leakage from and *Synechococcus* UTEX 625. Plant Cell and Environment **19,** 260–274.

Salon C, Canvin DT. 1997. HCO_3^- efflux and the regulation of intracellular C_i pool size in *Synechococcus* UTEX 625. Canadian Journal of Botany **75,** 290–300.

Sharkey TD, Berry JA. 1985. Carbon isotope fractionation of algae influenced by an inducible CO_2-concentrating mechanism. In: Lucas WJ, Berry JA, eds. *Inorganic carbon uptake by aquatic photosynthetic organisms*. Rockville: American Society of Plant Physiologists, 389–401.

Sherlock DJ, Raven JA. 2001. Interactions between carbon dioxide and oxygen in the photosynthesis of three species of marine red algae. Botanical Journal of Scotland **53,** 33–43.

Still CJ, Berry JA. 2003. Global distribution of C3 and C4 vegetation: carbon cycle Implications. Global Biogeochemical Cycles 17, 1006, pp. 6-1– 6–13. doi: 10.1029/2001GB001807

Sültemeyer D, Rinast K-A. 1996. The CO_2 permeability of the plasma membrane of *Chlamydomonas reinhartii*: mass spectrometric ^{18}O-exchange measurements from $^{13}C^{18}O_2$ in suspensions of carbonic anhydrase-loaded plasma-membrane vesicles. Planta **200,** 358–368.

Tazoe Y, von Caemmerer S, Badger MR, Evans JR. 2009. Light and CO_2 do not affect the mesophyll conductance to CO_2 diffusion in wheat leaves. Journal of Experimental Botany **60,** 2291–2301.

Tazoe Y, von Cammerer S, Estavillo GM, Evans JR. 2011. Using tunable diode laser spectroscopy to measure carbon isotope discrimination and mesophyll conductance to CO_2 diffusion dynamically at different CO_2 concentrations. Plant Cell and Environment **34,** 580–591.

Tcherkez G. 2013. Is the recovery of (photo)respiratory CO_2 and intermediates minimal? New Phytologist **198,** 334–338.

Tchernov D, Hassidim N, Luz B, Sukenik A, Reinhold L, Kaplan A. 1997. Sustained net CO_2 evolution during photosynthesis by marine microorganisms. Current Biology **7,** 725–728.

Tchernov D, Hassidim N, Vardi A, Luz B, Sukenik A, Reinhold L, Kaplan A. 1998. Photosynthesizing marine organism can constitute a source of CO_2 rather than a sink. Canadian Journal of Botany **76,** 949–953.

Tchernov D, Silverman J, Luz B, Reinhold L, Kaplan A. 2003. Massive light-dependent cycling of inorganic carbon between oxygenic between oxygenic photosynthetic microorganism and their surroundings. Photosynthesis Research **77,** 95–103.

Tortell PD, Reinfelder JR, Morel FMM. 1997. Active uptake of bicarbonate by diatoms. Nature **390,** 243–244.

Tortell PD, Payne C, Guegen C, Strzepek RF, Boyd PW, Rost B. 2008. Inorganic carbon uptake by Southern Ocean phytoplankton. Limnology and Oceanography **53,** 1266–1278.

Tsuchihira A, Hanba YT, Kato N, Doi T, Kawazu T, Maeshima M. 2010. Effect of overexpression of radish plasma membrane aquaporins on water-use efficiency, photosynthesis and growth of *Eucalyptus* trees. Tree Physiology **30,** 417–430

Uehlein N, Lovisolo C, Siefritz F, Kaldenhoff R. 2003. The tobacco aquaporin NtAQP1 is a membrane CO_2 pore with physiological functions. Nature **425,** 734–737.

Uehlein N, Otto B, Hanson DT, Fischer M, McDowell N, Kaldenhoff R. 2008. Function of *Nicotiana tabacum* aquaporins as chloroplast gas pores challenges the concept of membrane CO_2 permeability. The Plant Cell **20,** 648–657.

Van Hunnik E, Amoroso G, Sültemeyer D. 2002. Uptake of CO_2 and bicarbonate by intact cells and chloroplasts of *Tetraedron minimum* and *Chlamydomonas noctigama.* Planta **215,** 763–769.

Von Caemmerer S. 1989. A model of photosynthesising CO_2 assimilation and carbon-isotope discrimination in leaves of certain C_3-C_4 intermediates. Planta **178,** 463–474.

Von Caemmerer S, Ghanoum O, Pengelly JJL, Cousins AB. 2014. Carbon isotope discrimination as a tool to explore C_4 photosynthesis. Journal of Experimental Botany **65,** 3459–3470.

Walker NA, Smith FA, Cathers IR. 1980. Bicarbonate assimilation by freshwater charophytes and higher plants. I. Membrane transport of bicarbonate is not proven. Journal of Membrane Biology **12,** 241–256.

Wang Y, Spalding MJ. 2014. Acclimation to very low CO_2: contribution of limiting CO_2 inducible proteins, LCIb and LCIA, to inorganic carbon uptake in *Chlamydomonas reinhardtii.* Plant Physiology **166,** 2040–2050.

Warren CR. 2008. Stand aside stomata, another actor deserves centre stage: the forgotten role of the internal conductance to CO_2 transfer. Journal of Experimental Botany **59,** 1475–1487.

Winter K, Smith JAC, **eds.** 1996. Crassulacean Acid Metabolism: Biochemistry, Ecophysiology and Evolution. Berlin, Germany: Springer.

Winter K, Holtum JAM, Smith JAC. 2015. Crassulacean acid metabolism: a continuous or discrete trait? New Phytologist **208,** 73–78.

Yamano T, Sato E, Iguchi H, Fukuda Y, Fukuzawa H. 2015. Characterization of cooperative bicarbonate uptake into chloroplast stroma in the green alga *Chlamydomonas reinhardtii.* Proceedings of the National Academy of Science USA **112,** 7315–7320.

Young JD, Shastri A, Stephanopoulos G, Morgan JA. 2011. Mapping photoautotrophic metabolism with isotopically nonstationary ^{13}C flux analysis. Metabolic Engineering **13,** 656–665.