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Ecological imperatives for aquatic carbon dioxide concentrating mechanisms

Stephen C. Maberly[1*] & Brigitte Gontero[2]

[1]Lake Ecosystems Group, Centre for Ecology & Hydrology, Lancaster Environment Centre, Library Avenue, Bailrigg, Lancaster LA1 4AP UK. E-mail: scm@ceh.ac.uk

[2]Aix Marseille Univ, CNRS, BIP, UMR 7281, IMM, FR 3479, 31 Chemin J. Aiguier, 13 402 Marseille Cedex 20, France. E-mail: bmeunier@imm.cnrs.fr

*Correspondence: S.C. Maberly E-mail: scm@ceh.ac.uk; Fax: +44 1524 61536; Tel: +44 1524 595851.

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Highlight: The interactions between cellular processes and environmental conditions that determine whether or not there is an ecological advantage to operating a CCM in aquatic environments are explored. (27 words)

Abstract

In aquatic environments, the concentration of inorganic carbon is spatially and temporally variable and CO_2 can be substantially over-saturated or depleted. Depletion of CO_2 plus low rates of diffusion cause inorganic carbon to be more limiting in aquatic than terrestrial environments and the frequency of species with a CCM, and their contribution to productivity is correspondingly greater. Aquatic photoautotrophs may have biochemical or biophysical CCMs and exploit CO_2 from the sediment or the atmosphere. Though partly constrained by phylogeny, CCM activity is related to environmental conditions. CCMs are absent or down-regulated when their increased energy costs, lower CO_2 affinity or altered mineral requirements outweigh their benefits. Aquatic CCMs are most widespread in environments with low CO_2, high HCO_3^-, high pH and high light. Freshwater species are generally less effective at inorganic carbon removal than marine species but have a greater range of ability to remove carbon, matching the environmental variability in carbon availability. The diversity of CCMs in seagrasses and marine phytoplankton and detailed mechanistic studies on larger aquatic photoautotrophs are understudied. Strengthening the links between ecology and CCMs will increase our understanding of the mechanisms underlying ecological success and will place mechanistic studies in a clearer ecological context.

Keywords: Aquatic CCM, CO_2, inorganic carbon, macroalgae, macrophytes, photosynthesis, phytoplankton, seagrasses

Introduction

Photosynthesis on land contributes ~50% to the total global net primary productivity of about 105 Pg C y^{-1} (Field *et al.*, 1998) and is performed by approximately 300,000 species of terrestrial photoautotrophs (Kreft and Jetz, 2007), almost exclusively within the Plantae. About 275,000 (92%) of the terrestrial species perform C_3 carbon-fixation and contribute about 77% of terrestrial productivity (Still *et al.*, 2003). C_3 photosynthesis involves passive diffusion of CO_2 down a concentration gradient that is produced by carbon fixation by the primary carboxylating enzyme, ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco). In theory, this is all that is required to fix carbon, but Rubisco's low rates of catalysis, low affinity for CO_2 and ability to fix oxygen can lead to photorespiration, and limit rates of net photosynthesis (Laing *et al.*, 1974; Raven and Johnston, 1991; Giordano *et al.*, 2005).

While C_3 plants rely on 'pull down' of CO_2 by Rubisco fixation, some terrestrial plants have a biochemical CO_2 concentrating mechanism (CCM) that 'pushes in' CO_2 towards Rubisco, increasing the concentration of CO_2 around the active site and minimising photorespiration. This mechanism involves pre-fixation of carbon by the carboxylating enzyme phospho*enol*pyruvate carboxylase (PEPC), that is unaffected by oxygen and produces a four-carbon compound that is decarboxylated to generate CO_2 around Rubisco. Plants with a CCM based on C_4 carboxylation contribute the remaining 23% of terrestrial productivity. Two related types of C_4-based CCMs are found in terrestrial plants. In C_4 photosynthesis, there is an intra-cellular or inter-cellular spatial separation of C4 carboxylation and decarboxylation (Voznesenskaya *et al.*, 2001; Sage, 2002), while in crassulacean acid metabolism (CAM), there is a temporal separation of daytime C_4 de-carboxylation and night-time C_4 carboxylation. C_4 photosynthesis evolved independently at least 66 times and is present in about 8,100 species (about 2.7%) of terrestrial plants (Sage, 2016), while CAM also evolved in multiple lineages, and is found in 16,800 species (about 6% of terrestrial plants; (Silvera *et al.*, 2010)). These additional pathways have costs but can also confer ecological benefits. On land, plants with a CCM have an ecological advantage in sub-tropical, tropical and arid or saline environments where reduced stomatal conductance decreases water loss but also reduces the internal CO_2 concentration (Osborne and Freckleton, 2009; Sage, 2016).

Aquatic ecosystems contribute the remaining ~50% of global net primary productivity, which, unlike terrestrial productivity, is carried out by a large phylogenetic diversity of organisms. Although marine angiosperms, seagrasses, (72 species; (Les and Tippery, 2013)) and submerged freshwater angiosperms, macrophytes, (about 6000 species; (Cook, 1990)), can be very important local 'ecosystem engineers' they cover a low area of the globe and consequently these Plantae contribute relatively little to global productivity (van der Heijden and Kamenos, 2015). Instead, most aquatic primary production is performed by planktonic cyanobacteria and eukaryotic microalgae in the ocean with a small contribution from eukaryotic macroalgae (about 1 Pg C y^{-1}, 2% of ocean productivity (Field *et al.*, 1998) and about 0.17 Pg C y^{-1} contribution to carbon-sequestration (Krause-Jensen and Duarte, 2016)). The global number of photoautotrophic algal species (including cyanobacteria) is poorly constrained but might be in the region of 150,000 (Guiry, 2012; Mann and Vanormelingen, 2013). In contrast to terrestrial primary producers, CCMs are widespread in aquatic primary producers, make a major contribution to aquatic primary productivity and include the biochemical C_4 and CAM CCMs, found in their terrestrial counterparts, and also biophysical CCMs based on active uptake of HCO_3^- or CO_2 or both that are largely absent in land plants.

The aim of this review is to set the ecological context for aquatic CCMs. To do this, we outline the availability of inorganic carbon in water, summarise the strategies that aquatic photoautotrophs use to maximise carbon uptake, quantify the effectiveness (used herein to refer to the ability to exploit the in carbon reserves) in CCMs in different types of photoautotroph, examine the costs and benefits of operating a CCM and describe the ecological conditions where a CCM appears to increase fitness and creates an ecological advantage.

Availability of inorganic carbon in water

Concentration of CO_2 and O_2 at air-equilibrium

The solubility of CO_2 and O_2 are described by Henry's law and are independent of variables such as pH. At 15°C and standard pressure, the concentration of CO_2 in fresh water in equilibrium with an atmosphere containing 400 ppm CO_2, is ~18 mmol m^{-3} (Fig. 1A). In sea water with a salinity of 34, and the same temperature, the concentration will be ~20 % lower at ~15 mmol m^{-3}. Under these conditions, the concentration of oxygen will be 314 mmol m^{-3} in fresh water and ~19% lower in seawater at 256 mmol m^{-3}. In both fresh water and sea water, the solubility of CO_2 declines with temperature by about 2.3% °C^{-1} between 5 and 25 °C, while the solubility of O_2 declines by about 1.7% °C^{-1} over the same temperature range. Consequently, at equilibrium with the atmosphere, the molar ratio of CO_2 to O_2 declines from about 0.064 at 5°C to 0.053 at 25°C in both environments.

Rate of diffusion

A major difference between photosynthesis in water and air is the rate of gas diffusion which for CO_2 in water at 25°C is 2 10^{-9} m^2 s^{-1}, about 10,000 lower than in air (Raven, 1970; Zeebe, 2011). Since materials have to diffuse through the boundary layer that surrounds all cells, thalli or leaves in a fluid (Vogel, 1994), the external transport resistance to carbon fixation can be high

(Black *et al.*, 1981) particularly for larger objects in areas of low water velocity. Rates of O_2 diffusion in water are similarly lower than in air (Verberk *et al.*, 2011) and as a result rates of loss of O_2 out of a photosynthesising cell or tissue, will also be restricted by transport limitation, increasing internal O_2 concentrations and further promoting the tendency for photorespiration (Mass *et al.*, 2010). As a consequence, half-saturation concentrations for CO_2 for photosynthesis in water are frequently substantially above air-equilibrium, especially in the larger photoautotrophs. For example, half-saturation constants for CO_2 in freshwater macrophytes without a CCM, are about seven-times greater than air-equilibrium (Maberly and Madsen, 1998) while C_3 photosynthesis in air is only stimulated by about 30% at elevated CO_2 levels (Lloyd and Farquhar, 1996; Ainsworth and Long, 2005).

Forms of inorganic carbon in water

Unlike air, four forms of inorganic carbon exist in water. When CO_2 dissolves, a small proportion (<0.2%; (Stumm and Morgan, 2012)) reacts with water producing carbonic acid (H_2CO_3), which together with dissolved CO_2 comprises free CO_2. Carbonic acid can dissociate forming bicarbonate (HCO_3^-), and CO_2 can also react with water, or OH^- at high pH, to form HCO_3^- directly. Bicarbonate can dissociate further forming carbonate (CO_3^{2-}). Free CO_2, HCO_3^- and CO_3^{2-} in aggregate comprise the concentration of total dissolved inorganic carbon (C_T). The equilibria between the different forms of inorganic carbon is controlled by temperature, ionic strength and particularly pH. At 15°C in fresh water with the average global river ion concentration (ionic strength 1.1 mol m^{-3}; (Meybeck, 2003)), the first and second dissociation constants (pK_1' and pK_2' representing the pH where the concentrations of CO_2 and HCO_3^- and HCO_3^- and CO_3^{2-} are equal) are 6.41 and 10.38 respectively. In sea water at the same temperature they are 5.95 and 9.11; which is 0.46 and 1.27 pH units lower than fresh water for pK_1' and pK_2' respectively (Fig. 1B). As a consequence, CO_3^{2-} ions are a larger proportion of C_T at a given pH in sea water compared to fresh water.

Although under many circumstances, the different forms of inorganic carbon can be considered to be in equilibrium, the kinetics of hydration and dehydration between CO_2 and HCO_3^- are relatively slow and can be exceeded by high rates of CO_2 or HCO_3^- exchange per unit volume. This can cause the concentration of CO_2 or HCO_3^- to be pulled out of equilibrium from the concentration set by the physico-chemistry. The enzyme carbonic anhydrase acts to maintain chemical equilibrium between CO_2 and HCO_3^- and is widely involved in different CCMs (Raven, 1995).

Variation in inorganic carbon availability

Alkalinity is the sum of bases formed from weak acids, roughly equivalent to acid neutralising capacity and usually mainly represented by HCO_3^-. Variable geology leads to a variable alkalinity in fresh waters (Meybeck, 2003). It can exceed 1000 equiv m^{-3} in soda lakes where evaporation, rather than outflow, is the major process of water loss (Talling, 1985). It can also be very low or negative (net acidity) in acid sites affected by acid deposition (Battarbee, 1990), at peaty sites with a high concentration of organic acids, or at sites in the vicinity of volcanic activity or acid mine drainage where sulphide oxidation generates protons (Evangelou and Zhang, 1995; Pedrozo *et al.*, 2001). Based on the GLORICH database of river chemistry, (Hartmann *et al.*, 2014) and site average values for 5303 streams or rivers with a Strahler order of 3 or greater, alkalinity as a proxy for the concentration of bicarbonate, had a median concentration of 1,559 mequiv m^{-3} (Table 1). In contrast, the contemporary ocean has a comparatively constant alkalinity of about 2,300 mequiv m^{-3}.

At air-equilibrium, pH can vary from less than 2 in highly acid sites, to around 5 to 8 in lakes with low to moderate alkalinity, to over 10 in lakes with very high alkalinity (Fig. 1C) (Talling, 1985). At 15°C, the air-equilibrium pH of sea water is about 8.1 (equilibrium with an atmosphere of 400 ppm) and the concentration of CO_2 represents about 0.7% of the dissolved inorganic carbon, while in fresh water, at a slightly high equilibrium concentration, it can vary between 100% of the inorganic carbon in acid sites, to less than 0.2% at sites with a high alkalinity (i.e. > 10 equiv m^{-3})

Overlying the variation in pH at equilibrium with the atmosphere, biological production of CO_2 by respiration or removal of CO_2 or HCO_3^- by photosynthesis, can alter C_T and pH and consequently the concentration and proportions of the different forms of inorganic carbon. Accordingly, the concentration of CO_2 and the other forms of inorganic carbon can be under- or over-saturated compared to air-equilibrium (Fig. 2). Globally, annual average concentrations of CO_2 are oversaturated in most lakes and rivers (Cole *et al.*, 1994; Raymond *et al.*, 2013). This results from microbial activity and photodegradation of organic carbon produced on land (Sobek *et al.*, 2007) within the water body and also from lateral transfer of CO_2-rich water produced by microbial breakdown of terrestrial organic carbon within the catchment (Maberly *et al.*, 2013). Analyses of large-scale global datasets have shown median values for CO_2 concentration of 1598 μatm in streams and rivers and 736 μatm in lakes and reservoirs (Table 1) (Raymond *et al.*, 2013). At an assumed mean temperature of 15°C this is roughly equivalent to median CO_2 concentrations of 73 mmol m^{-3} in streams and rivers and 34 mmol m^{-3} in lakes and reservoirs. Concentrations of CO_2 are higher in tropical regions than in temperate or boreal regions (Lauerwald *et al.*, 2015). Given the values of alkalinity for rivers from the GLORICH database noted above and in Table 1, median values of CO_2 are about 20-times lower than median values of HCO_3^- in rivers and, assuming similar alkalinity in lakes and rivers, about 46-times lower than the median concentration of HCO_3^- in lakes. Particularly high concentrations of CO_2 can also be generated in heterotrophic environments, especially those with restricted access to the atmosphere. For example, respiration can produce CO_2 concentrations over 2,000 mmol m^{-3} at depth in a lake (Casper *et al.*, 2000), between 300 and 5,000 mmol m^{-3} in aquatic sediments (Madsen *et al.*, 2002), over 400 mmol m^{-3} in groundwaters (Demars and Tremolieres, 2009; Stets *et al.*, 2009; Maberly *et al.*, 2015) and for Swedish and Finnish lakes under ice, median values of 160 to 340 mmol m^{-3} and maximum values of 580 to 870 mmol m^{-3} (Denfeld *et al.*, 2016).

Although lakes, and especially rivers, tend to be over-saturated with CO_2 at an annual scale, during periods of high photosynthetic demand, the concentration of CO_2 can become depleted below air-equilibrium in many aquatic systems. Even lakes which are oversaturated with CO_2 on an annual scale can experience substantial depletion in CO_2 during productive periods ((Maberly, 1996) Fig. 2) and the same can be true in rivers (Jarvie *et al.*, 2017). The least variable aquatic systems in terms of CO_2 are those of low productivity such as the open ocean where pH is fairly constant at about 8 to 8.1 (Hofmann *et al.*, 2011) and the CO_2 concentration is always close to air equilibrium. However, at decadal scales as atmospheric CO_2 has increased, the air-equilibrium concentrations of CO_2 has also increased and ocean pH has decreased (Doney *et al.*, 2009). In the coastal ocean, with higher productivity and in dense beds of macroalgae with high demand, pH can become elevated and the CO_2 concentration depleted below air-equilibrium (Delille *et al.*, 2000; Middelboe and Hansen, 2007; Hofmann *et al.*, 2011; Cornwall *et al.*, 2013; Krause-Jensen *et al.*, 2016). There can also be substantial daily and semi-diurnal changes in CO_2 concentration over 24 hours and extreme depletion in sites with extremely high biomass such as rockpools (Maberly, 1992). In productive lakes, large summer populations of phytoplankton, especially cyanobacteria (e.g. (Talling, 1976; Maberly, 1996; Ibelings and Maberly, 1998)), or dense macrophyte beds (Van *et al.*, 1976) can reduce photic-zone concentrations of CO_2 close to zero for extended periods in the summer when demand outstrips supply ((Maberly, 1996); Fig. 2). Under these circumstances, a CCM is essential for continued photosynthesis, especially since high concentrations of oxygen (up to 4-fold air equilibrium, (Van *et al.*, 1976)), normally co-occur with carbon-depletion. Furthermore, under extreme carbon-depletion, even HCO_3^-, the uptake of which is a widespread aquatic CCM, can start to become depleted (Fig. 2C).

An example calculation illustrates the potential mismatch between rates of transport of CO_2 across the air-water interface and the rates of biological transformation of inorganic into organic carbon. Using the data and calculations for 1993 in a productive UK lake, Esthwaite

Water, (Maberly, 1996) and assuming a subsurface boundary layer thickness of 300 μm, an atmospheric CO_2 partial pressure of 360 ppm and allowing for chemical enhancement, the maximum rate of CO_2 influx across the air-water interface is 0.8 μmol m^{-2} s^{-1} and the average summer values are about 0.2 μmol m^{-2} s^{-1}. If one assumes a surface mixed layer thickness of 5 m, a chlorophyll *a* concentration of 40 mg m^{-3} (the summer mean for 1993 in Esthwaite Water) and an average rate of photosynthesis of 100 μmol mg^{-1} Chl*a* h^{-1}, the demand for carbon per unit surface area in the upper mixed layer will be about 5.6 μmol m^{-2} s^{-1}, which is nearly 30-times the average rate of CO_2-influx and about 7-times the maximum rate of CO_2-influx. In these types of systems as a consequence, the concentration of CO_2 can routinely vary 10-fold in 24 hours, driven by the light-dark cycle.

Biochemical, biophysical and morphological responses to CO_2 availability

Aquatic photoautotrophs respond to variable and often limiting supply of CO_2 and HCO_3^- in ways that involve Rubisco characteristics, biochemical pathways, physiological processes and morphological and anatomical changes.

Relationship between Rubisco kinetics and presence of a CCM

The form of Rubisco and its kinetic properties differ among photoautotrophs and can be seen, in part, as an adaptation to CO_2 availability. Many forms of Rubisco exist as a consequence of the diverse evolutionary origin of oxygenic photoautotrophs (see Table 1 and Fig. 3 in (Tabita *et al.*, 2008). The Forms IA (α-cyanobacteria), IB (β-cyanobacteria, glaucophytes, green algae, euglenophytes (Zahonova *et al.*, 2016) chlorarachniophytes and embryophytes) and ID (red algae, cryptophytes, haptophytes and heterokonts) are hexadecamers made up of eight large and eight small subunits, while the Form II of dinophyceae (and anoxygenic photosynthetic bacteria) (Morse *et al.*, 1995) are either dimers or multimers of the large subunit, although dinophytes with tertiary endosymbiosis may also contain chloroplasts with Form IB and ID (Tamura *et al.*, 2005; Minge *et al.*, 2010). The ID isoform, present in the ecologically important coccolithophores and diatoms within the heterokonts, differs substantially in amino acid sequence from the well-studied IB isoform (Clement *et al.*, 2017b).

The structural types of Rubisco do not match their kinetic properties however. The average Rubisco specificity factor, τ, defined as V_cK_o/V_oK_c (where V_c and V_o are the maximal velocities of carboxylation and oxygenation, respectively, and K_c and K_o the Michaelis constants for CO_2 and O_2), represents the propensity to catalyze the carboxylation *versus* the oxygenation reactions that lead to photorespiration. Although C_3 and C_4 land plants, diatoms and coccolithophores have different forms of Rubisco, the specificity factor is similar at about 80 to 90 while those from cyanobacteria and dinoflagellates are lower and those from red algae twice as high (Tortell, 2000; Young *et al.*, 2016). Conversely, although diatoms and red algae have Form ID Rubisco, their specificity factors are very different (Tortell, 2000). Within C_3 land plants, the Rubisco specificity factor is higher in species in environments where water supply is limited which is consistent with lower internal concentration of CO_2 in relation to stomatal closure (Galmes *et al.*, 2005). The K_c for Rubisco is also variable with average values of about 31 mmol m^{-3} in green algae (3 species), 16 and 13 mmol m^{-3} in bryophytes and ferns (two species each), about 10 mmol m^{-3} in C_3 land plants (23 species) and red algae (five species) and 47 mmol m^{-3} in diatoms (ten species) ((Young *et al.*, 2016) their SI and Table 1). Thus these values bracket typical air-equilibrium CO_2 concentrations (at 25°C about 11 and 14 mmol m^{-3} in sea and fresh water respectively, Fig. 1), reinforcing the benefit of a CCM for species that have a Rubisco with a poor affinity for CO_2. In cyanobacteria, for which a CCM has been shown in all studied photoautotrophic species, Rubisco has an even lower affinity for CO_2 which is often > 200 mmol m^{-3} (Moroney and Somanchi, 1999) and as high as 750 mmol m^{-3} in low light adapted strains of *Prochlorococcus marinus* (Scott *et al.*, 2007), concentrations that are rarely found in photic aquatic environments. Therefore, the kinetic properties of Rubisco appear to be related to CCM activity. For example, many red macroalgae such as *Lemanea* and *Batrachospermum* in fresh water and *Lomentaria* and *Delesseria* in marine systems with an assumed high specificity factor, lack a CCM (Raven and Beardall, 1981; Maberly, 1990). Tortell showed in an analysis of seven phytoplankters from different phylogenetic groups that there was an inverse relationship between τ and the extent of a CCM (Tortell, 2000). Thus carbon uptake can either be supported by Rubisco with a high specificity for CO_2 or Rubisco with a lower affinity compensated for by a CCM that is more effective in terms of concentrating CO_2.

Responses to CO_2 availability not involving a CCM

Not all photoautotrophs have a CCM (Raven *et al.*, 2005) and not all aquatic systems have low concentrations of CO_2, at least as an annual mean as outlined above (Table 1). Thus, plants with an 'avoidance strategy' (*sensu* Klavsen *et al.* (2011)), may simply grow in environments where CO_2 is sufficient for their photosynthetic needs and therefore do not require a CCM. In the 'exploitation strategy', morphological and anatomical adaptations allow sources of CO_2 that are more reliable than the bulk water to be exploited. Concentrations of CO_2 within the sediment are high as photosynthesis is absent and organic matter is mineralised generating CO_2. Many freshwater macrophytes have extensive lacunae that can be continuous from root to shoot (Sculthorpe, 1967), providing a transport route for CO_2 from the sediment to the leaves. The possibility of this path as a carbon source was suggested in the older literature but first shown experimentally for the short isoetid *Lobelia dortmanna* (Wium-Andersen, 1971). This exploitation strategy is present in most isoetids (Winkel and Borum, 2009) but diffusion resistance within the lacunae limits its ability to supply CO_2 to species of short stature and consequently in taller species such as *Myriophyllum spicatum*, root uptake contributes little to total carbon uptake (Loczy *et al.*, 1983). In the case of *Isoetes australis*, achlorophyllous leaf bases within the sediment also act as sources of CO_2-entry to the internal lacunae (Pedersen *et al.*, 2011). Borum et al. (Borum *et al.*, 2015) showed that oxygen concentrations in the buried base of the shoot of the seagrass *Zostera polychlamys* were 29% higher when the CO_2 concentration around the shoot was saturating compared to 9 mmol m^{-3}. This implies some diffusion of gases between root and shoot but the extent to which this supplies CO_2 to leaves is likely to be low given the diffusion distance; more work is warranted on this. It has also been suggested that early terrestrial lycopsids may also have benefitted from tapping into elevated CO_2 within the sediment (Green, 2010).

Freshwater macrophytes form part of a cline from dry land with complete reliance on photosynthesis in air, to submergence with complete reliance on photosynthesis in water. This gradation also reflects the evolutionary invasion of terrestrial embryophytes into water (Du and Wang, 2014). The more constant supply of CO_2 in the atmosphere is an opportunity for submerged plants to maximise carbon uptake. In the freshwater macrophyte *Callitriche cophocarpa* which lacks a CCM, growth rates were stimulated three-fold when floating leaves had access to atmospheric CO_2 and submerged leaves were at air-equilibrium (Madsen and Breinholt, 1995). The benefit of atmospheric CO_2 to amphibious freshwater plants has been widely demonstrated and promotes flowering in *Nuphar lutea* and *Callitriche hamulata* (= *C. intermedia* (Grainger, 1947), increases soluble carbohydrate reserves in *Hippuris vulgaris* (Janauer and Englmaier, 1986) and stimulates photosynthesis in *Stratiotes aloides* (Prins and Deguia, 1986).

The nature of aquatic CCMs

Many types and mechanisms of CCM exist in aquatic photoautotrophs. Several rely on production of particular localized environments by special structures such as the carboxysome, pyrenoid, charosome or transfer cells, of which latter, more than one type is found in seagrasses (Larkum et al THIS ISSUE). Some aquatic species possess biochemical CCMs analogous to those in terrestrial plants. The best known is that of the freshwater angiosperm *Hydrilla verticillata* that was studied by George Bowes and his group for over four decades (Bowes *et al.*, 2002; Bowes, 2011). This species is a member of the monocotyledon family Hydrocharitaceae, lacks Kranz anatomy, but possesses a facultative C_4 metabolism that is induced when CO_2 is limiting (Holaday and Bowes, 1980). Spatial separation between carboxylation and decarboxylation is achieved by PEPC being located in the cytoplasm while decarboxylation by NADP malic enzyme (NADP-ME) occurs with Rubisco in the chloroplast (Reiskind *et al.*, 1989; Magnin *et al.*, 1997; Reiskind *et al.*, 1997). Although also lacking Kranz anatomy, similar C_4 metabolism has been found in other species of the Hydrocharitaceae including *Egeria densa* (Casati *et al.*, 2000) and *Ottelia alismoides* (Zhang *et al.*, 2014). Unlike the two other species, *O. alismoides* appears to use NAD-ME rather than NADP-ME as the decarboxylating enzyme (Zhang *et al.*, 2014), (Shao *et al.*, 2017). There is less detailed evidence for C_4 metabolism in marine angiosperms (seagrasses). It is possible that *Cymodocea nodosa*, *Halophila stipulacea* and *Thalassia testudinum* possess C_4 metabolism (Beer *et al.*, 1980; Koch *et al.*, 2013) but further investigations are required. Within the marine macroalgae, there is good evidence for an ancient form of C_4 metabolism in the coenocytic, chlorophyte alga *Udotea flabellum* that is based on phospho*enol*pyruvate carboxykinase (PEPCK) which acts as a carboxylase in the cytosol and a decarboxylase in the chloroplast (Reiskind *et al.*, 1988; Reiskind and Bowes, 1991). There are hints of possible C_4 metabolism in the brown alga *Dictyota guineënsis* and the red alga *Palisada perforata* (=*Laurencia papillosa*) based on activities of PEPC and PEPCK compared to Rubisco (Holbrook *et al.*, 1988; Koch *et al.*, 2013); see also (Raven and Giordano, in press). The marine diatom *Thalassiosira weissflogii* has been proposed to possess single-cell C_4 metabolism (Reinfelder *et al.*, 2000; Reinfelder, 2011), although the evidence for this is not very conclusive (Clement *et al.*, 2017a) (Clement *et al.*, 2017b). Although not fully resolved, recent studies, on *T. pseudonana* (Tanaka *et al.*, 2014; Clement *et al.*, 2016; Clement *et al.*, 2017b) and *P. tricornutum* (Haimovich-Dayan *et al.*, 2013; Yang *et al.*, 2016) (Clement *et al.*, 2017a) using different approaches, suggest these species do not possess C_4 metabolism.

Aquatic CAM was first shown in the freshwater lycophyte *Isoetes howellii* (Keeley, 1981; Keeley, 2014) and has been recorded in all species of *Isoetes* that have been studied (Keeley, 1998). It is also found in other freshwater angiosperms including *Littorella uniflora* (Madsen, 1987b; Robe and Griffiths, 2000), *Crassula helmsii* (Newman and Raven, 1995), *Deinostema violaceum* (Yin *et al.*, in press) and *O. alismoides* (Zhang *et al.*, 2014), (Shao *et al.*, 2017). The presence of CAM in marine macroalgae is unclear (Koch *et al.*, 2013). Low amplitude diel changes in acidity have been reported in *Ascophyllum nodosum* (Johnston and Raven, 1987) and some other fucoid algae (Keeley, 1998) but more research is required to determine whether or not this constitutes CAM activity.

The remainder of aquatic CCMs are based on biophysical active transport of CO_2, HCO_3^- or both (Raven and Beardall, 2016). The most widespread CCM in aquatic plants, is based on access to HCO_3^- and is likely to depend on more than one mechanism. One, found notably in leaves of the monocotyledon genera *Potamogeton*, *Elodea, Egeria* and *Hydrilla* involves polar leaves. Protons are excreted at the abaxial surface, converting bicarbonate to CO_2 some of which will diffuse into the leaf, while at the adaxial surface net hydroxide excretion leads to the deposition of marl (Steemann Nielsenn, 1947; Prins *et al.*, 1980; Prins *et al.*, 1982; Prins and Elzenga, 1989). A similar process occurs in charophytes where acid and alkaline bands are present along the axis of giant cells (Lucas and Smith, 1973).

Some freshwater angiosperms that can use HCO_3^-, such as the dicotyledon genera *Myriophyllum* or *Ranunculus*, lack spatial patterns of acid and alkaline zones and instead H^+-HCO_3^- co-transport has been proposed (Prins and Elzenga, 1989). In marine macroalgae, also without evidence of acid or alkaline zones, there is a range of different proposed mechanisms (Raven and Hurd, 2012). These include bicarbonate transporters in *Ectocarpus siliculosus* (Gravot *et al.*, 2010), anion exchange proteins in *Macrocystis pyrifera* (Fernandez *et al.*, 2014) and proton extrusion acting with external carbonic anhydrase (but not involving acid and alkaline zones) in *Saccharina latissima* (= *Laminaria saccharina*) (Axelsson *et al.*, 2000). The green alga *Ulva lactuca* possesses two mechanisms to use HCO_3^-, one based on carbonic anhydrase and external acidification of the boundary layer at seawater pH, again without discernible acid and alkaline zones, and one based on an anion transporter that is up-regulated after incubation at high pH (Axelsson *et al.*, 1995).

CCMs in cyanobacteria are among the best studied of any group of photosynthetic organisms. Active transporters of CO_2 and HCO_3^- produce a large internal pool of HCO_3^- that is converted to CO_2 around Rubisco within specialized structures that restrict leakage, carboxysomes (Price *et al.*, 2008; Price, 2011). *Chlamydomonas reinhardtii* has the best-studied eukaryotic CCM that involves HCO_3^- transporters located at the plasmalemma and the outer stromal membrane that generate high concentrations of HCO_3^- in the chloroplast stroma (Wang *et al.*, 2011). There are at least twelve genes present encoding carbonic anhydrases in different locations (Moroney *et al.*, 2011). The alpha CA in the acid thylakoid lumen (CA3) is believed to increase the rate of conversion of HCO_3^- to CO_2 which can then diffuse out to the surrounding pyrenoid which is the location of Rubisco. A beta carbonic anhydrase (CA6) in the stroma has been suggested to trap CO_2 that has diffused out of the lumen and bypassed the pyrenoid by speeding its conversion back to HCO_3^- (Moroney *et al.*, 2011).

In diatoms, several biophysical CCM mechanisms are involved including bicarbonate active transporters(Matsuda *et al.*, 2011; Nakajima *et al.*, 2013) and carbonic anhydrase enzymes (Harada *et al.*, 2005; Trimborn *et al.*, 2009; Crawfurd *et al.*, 2011; Hopkinson *et al.*, 2011; Hopkinson *et al.*, 2013; Clement *et al.*, 2016); (Clement *et al.*, 2017a). The nature of the CCM has also been studied in other marine phytoplankton including chlorophytes and other chromalveloates from the dinoflagellates, haptophytes (including coccolithophores) and eustigmatophytes (Huertas *et al.*, 2000; Colman *et al.*, 2002; Rost *et al.*, 2003; Dason *et al.*, 2004; Hoins *et al.*, 2016; Kottmeier *et al.*, 2016). These and other studies (Clement *et al.*, 2017a) (Riebesell *et al.*, 1993; Clark and Flynn, 2000) show that there is a large diversity in ability of marine phytoplankton to utilize the carbon reserves.

Regulation of CCMs by environmental conditions

It has been known for a long time that algae are phenotypically plastic and their photosynthetic characteristics can be altered by environmental conditions e.g. (Briggs and Whittingham, 1952). In microalgae where this regulation has been best studied and for which a substantial literature exists, a CCM is rapidly up-regulated at low CO_2 and down-regulated at high CO_2 (Giordano *et al.*, 2005). Similar regulation of HCO_3^- use occurs in freshwater macrophytes (Sand-Jensen and Gordon, 1986; Madsen *et al.*, 1996; Adamec, 2009). In *Myriophyllum alterniflorum* there are phenotypic differences between populations from low and high alkalinity sites which largely disappear after incubation in standard conditions (Maberly and Madsen, 2002a). C_4 metabolism in freshwater macrophytes is up-regulated when inorganic carbon is limiting in *H. verticillata* and *E. densa* (Casati *et al.*, 2000; Bowes, 2011) but appears to be constitutive in *O. alismoides* (Zhang *et al.*, 2014). In contrast, C_4 metabolism is present in leaves of *Eleocharis vivipara* when in air but absent when in water (Ueno, 1998; Murphy *et al.*, 2007). CAM activity in freshwater macrophytes is up-regulated at low CO_2 and high light (Madsen, 1987a; Robe and Griffiths, 1990; Baattrup-Pedersen and Madsen, 1999; Klavsen and Maberly, 2010) (Shao *et al.*, 2017) and can also be down-regulated when leaves are exposed to the more constant supply of CO_2 in air (Aulio, 1986; Robe and Griffiths, 2000; Yang and Liu, 2015). In the halotolerant *Dunaliella salina*, high salinity (2.22 kmol m^{-3} NaCl) promoted a CCM, presumably because high salinity reduces the solubility of CO_2 (Booth and Beardall, 1991).

Low light can lead to a reduction in CCM activity in eukaryotic and prokaryotic microalgae (Beardall, 1991; Beardall and Giordano, 2002; Raven and Beardall, 2014). There is evidence of regulation of CCMs in microalgae by variation in the concentration and form of nitrogen and the concentration of phosphorus (Beardall and Giordano, 2002; Raven and Beardall, 2014). However, for both elements, both increases and decreases in inferred activity of a CCM in response to limitation have been recorded (Raven and Beardall, 2014).

Costs & Benefits of CCMs

The fact that CCMs occur in some but not all species, are frequently down-regulated when inorganic carbon is not limiting (Giordano *et al.*, 2005) and can be lost in microalgae evolving over 1000 generations at high CO_2 (Collins *et al.*, 2006) indicates that their operation incurs costs as well as conferring benefits.

Energy costs

By definition, a CCM involves an energy cost because it generates a concentration of CO_2 at the active site of Rubisco above that which could be achieved by passive processes. However, Sage and Khoshravesh (Sage and Khoshravesh, 2016) point out that locating photorespiratory and respiratory release of CO_2 close to Rubisco may act as a passive CCM that can mitigate, rather than overcome, the consequences of Rubisco's properties. An active CCM will incur energy costs for investing in catalytic machinery and running the CCM (Raven and Lucas, 1985). This has ecological relevance because light is often, although not always, a limiting resource for aquatic photoautotrophs because of the relatively high rates of attenuation in aquatic environments (Maberly, 2014). A detailed calculation of the theoretical minimum energy (photon) running costs has been undertaken by (Raven *et al.*, 2014) as mol photons absorbed per mol carbon fixed. They estimated that when a CCM is absent, the minimum photon cost will be between 9.92 and 9.96. Assuming that no leakage of CO_2 is occurring out of the site where it is accumulated, the minimum photon cost when CCM is active varies between 9.25 and 10 depending on the precise CCM. Assuming a leakage rate equivalent to the rate of photosynthesis, the photon cost increases to between 9.5 and 11 mol photons absorbed per mol carbon fixed (Raven and Beardall, 2016). The additional costs of producing the CCM machinery are more uncertain and complicated by trade-offs with other resources such as nitrogen and phosphorus.

A CCM is present in psychrophilic marine diatom communities from the Western Antarctic Peninsula, including species such as *Fragilariopsis cylindrus*. However, it operates with a relatively low energy cost (Kranz *et al.*, 2015) because at 0°C the Michaelis constant for Rubisco (K_c) is only 15 mmol CO_2 m^{-3} while the air equilibrium the CO_2 concentration is 25 mmol m^{-3} (Young *et al.*, 2015). Even though the ambient CO_2 concentration had been reduced to about 6 mmol m^{-3} in a bloom, the diatoms were nearly saturated with inorganic carbon by the CCM at a low energy cost (Kranz *et al.*, 2015; Young *et al.*, 2015).

Affinity costs

A less recognised cost of operating at least some types of CCM relates to the affinity for CO_2. In a review of the kinetics of CO_2 uptake from a range of freshwater macrophytes, it was shown that the $K_{½}$ for CO_2 for species able to use HCO_3^- as well as CO_2 was about 210 mmol m^{-3} but only 108 mmol m^{-3} for species restricted to CO_2 (Maberly and Madsen, 1998). The slope of CO_2-uptake per CO_2 concentration was similarly greater in species restricted to CO_2. Using experiments in different gas mixtures, including helium, it was shown that species with the ability to use HCO_3^- have a higher internal resistance to CO_2 uptake than species restricted to CO_2 (Madsen and Maberly, 2003). This also occurs in other types of photoautotrophs; the permeability of plasmalemma from *Chlamydomonas reinhardtii* grown at high CO_2 is about 1.7-times greater than those grown at low CO_2, consistent with these differences (Raven and Beardall, 2016). A high permeability is obviously advantageous in a species restricted to CO_2 but if a species is concentrating CO_2 internally with a CCM, a high permeability runs the risk of futile cycling. The different affinities of macrophytes with and without an ability to use HCO_3^- has a clear potential ecological significance in shallow water where light energy is high.

Other costs

There are other largely unquantified costs, or at least trade-offs, involved in the operation of a CCM. These involve a different element requirement when a CCM is present or absent (Raven and Johnston, 1991). For example, the enzyme carbonic anhydrase is ubiquitously up-regulated along with a CCM which will incur some attendant cost including those for the uptake of elements such as Zn, Co or Cd that can be present at low concentration, especially in the open ocean (Lane and Morel, 2000). In marine phytoplankton, N and P quotas also vary with CO_2 concentration (Reinfelder, 2012) but the extent to which these incur a cost or benefit is poorly constrained.

Benefits

The benefits of operating a CCM have been alluded to and include reducing rates of photorespiration, increasing rates of photosynthesis when CO_2 is limiting and extending the amount of carbon that is accessible to photosynthesis during carbon depletion. An example of these benefits is shown in Fig. 3 where rates of carbon uptake for three species of the freshwater macrophyte *Myriophyllum* which vary in the presence and effectiveness of their CCM are compared. *M. verticillatum* lacks a CCM, being reliant on CO_2 alone, and had a CO_2 compensation point of 3.2 mmol m^{-3} allowing it to remove only 4% of the available inorganic carbon. *M. alterniflorum* and *M. spicatum* were able to use CO_2 and HCO_3^- and while *M. alterniflorum* could remove 51% of the available inorganic carbon, *M. spicatum* was even more effective and removed 80% of the available carbon. Rates at air-equilibrium, ~15 mmol m^{-3}, as a percentage of those at 200 mmol m^{-3}, which was not quite saturating, were 3%, 20% and 34% in *M. verticillatum*, *M. alterniflorum* and *M. spicatum* respectively (Fig. 3B), showing that the CCM based on HCO_3^- -use increased rates of photosynthesis at air-equilibrium in addition to increasing the pool of available inorganic carbon. For two of these species of *Myriophyllum*, these photosynthesis differences have recently been shown to translate to benefit growth at low

CO_2: *M. spicatum* growth was much less affected by CO_2 depletion than *M. verticillatum* (Dulger and Hussner, 2017).

Frequency and extent of CCMs in different types of photoautotroph

In addition to phenotypic plasticity in the activity of CCMs within a given species, there are genotypic differences in the effectiveness of CCMs to exploit the inorganic carbon reserves among different species which together generate a gradient in ability to deplete inorganic carbon in freshwater macrophytes and phytoplankton (Talling, 1976; Allen and Spence, 1981). Fig. 4 presents the compiled published and unpublished results for different types of aquatic photoautotrophs on the extent to which inorganic carbon can be removed from water based on pH-drift experiments. Percent carbon availability is calculated from the calculated or measured concentration of C_T at the end of a drift compared to the alkalinity of the medium. There is a wide range of ability to exploit the inorganic carbon reserves within each group, with species without a CCM only being able to remove a small fraction of the available carbon compared to species with an effective CCM. The seagrasses and marine phytoplankton had the highest median ability to remove inorganic carbon and the freshwater macrophytes clearly had the lowest (Fig. 4). However, freshwater macrophytes, freshwater phytoplankton and marine macroalgae had the largest range in ability to remove inorganic carbon, while the seagrasses (although with a limited number of species tested) and marine phytoplankton had the lowest range. Thus, broadly comparing marine and freshwater photoautotrophs, the marine photoautotrophs had a 1.7-fold greater median ability to extract inorganic carbon while the freshwater photoautotrophs had a 1.4-fold greater range in ability to extract inorganic carbon.

The lower median capability of freshwater macrophytes to exploit the reserves of inorganic carbon is consistent with their alternative strategies for acquiring inorganic carbon described above. Thus, only 50 species of the 110 tested (45%) are able to use HCO_3^- (Fig. 5A). Four species perform C_4 photosynthesis and 9 species perform CAM based on diel acidity changes. All the species with C_4 also use HCO_3^- while only two species, *Ottelia alismoides* (Zhang *et al.*, 2014) and possibly *Scirpus subterminalis* (Beer and Wetzel, 1981) and *Vallisneria spiralis* (Yin *et al.*, in press) combine both CAM and HCO_3^- use. *O. alismoides*, appears, uniquely, to combine three different types of CCM, HCO_3^- - use, C_4 and CAM (Shao *et al.*, 2017). Fifty two species have no apparent CCM. Of these, 69 % have access to CO_2 from the atmosphere, sediment or grow in environments where CO_2 is likely to be high locally (Fig. 5B). In contrast, only about 24% of species with an ability to use HCO_3^- have alternative strategies, and these largely comprise C_4 metabolism and access to atmospheric CO_2.

Most groups of eukaryotic algae and aquatic plants contain species with and without CCMs (Raven *et al.*, 2005). Nevertheless, phylogenetic differences underlie some of the variation in ability to remove inorganic carbon. Of particular note for their apparently uniform absence of a CCM is the group of heterokont algae, the Chrysophyceae *sensu latu* (= Synurophyceae and Chrysophyceae). No species studied from this group has been shown to be able to use HCO_3^- and there is no evidence for the operation of a CCM (Saxby-Rouen *et al.*, 1998; Maberly *et al.*, 2009; Bhatti and Colman, 2011). There are no clearly attested freshwater bryophytes or lycophytes with a CCM (Spence and Maberly, 1985) apart from in one experiment with *Fontinalis antipyretica* (Penuelas, 1985), and a possible CCM in an aquatic hornwort (Bain and Proctor, 1980), although terrestrial species from this group do have a CCM (Villarreal and Renner, 2012). Within the freshwater macrophyte genus *Callitriche*, most species lack a CCM but two of the three species within the subgenus Pseudocallitriche (Philbrick and Les, 2000), *C. hermaphroditica* (Maberly and Madsen, 2002b) and *C. truncata* (Whitney & Maberly unpublished) can also use HCO_3^-; the third species, has not yet been tested. Within the prokaryotes, all tested species of cyanobacteria appear to possess a CCM which compensates for the low affinity of their Rubisco enzyme. This is also the case for a culture of *Merismopedia glauca*, collected from the New Forest, England (Maberly, unpublished), which can grow in eutrophic lakes e.g. (Yamamoto and Shiah, 2012) but the genus contains species such as *M. tenuissima* that are often dominant in acid sites and absent when pH is increased by liming (Anderson *et al.*, 1997). Work is required on *M. tenuissima* to determine if this acid-tolerant cyanobacterium possesses a CCM.

Environmental conditions and CCM activity: towards establishing inorganic carbon as an ecological factor

In some circumstances, inorganic carbon can limit the primary productivity of marine phytoplankton (Riebesell *et al.*, 1993; Hein and Sand-Jensen, 1997; Clark and Flynn, 2000), freshwater phytoplankton (Ibelings and Maberly, 1998; Jansson *et al.*, 2012), marine macroalgae (Holbrook *et al.*, 1988), seagrasses (Borum *et al.*, 2015) and freshwater macrophytes (Madsen and Maberly, 1991). Given this and the environmental variability in inorganic carbon concentrations and other resources and the wide variability in abilities of aquatic photoautotrophs to acquire inorganic carbon, it is likely that inorganic carbon is one of the environment factors that controls the ecological distribution of aquatic photoautotrophs. Some of the differences between marine and freshwater photoautotrophs in Fig. 4 can be accounted for, in part, by the availability of inorganic carbon in the respective environments. The marine environment has a nearly uniform concentration of HCO_3^- and a lower variability in

CO_2 concentrations and CO_2 sources than freshwaters, increasing the benefit of using HCO_3^- but not placing a premium on a highly effective CCM. In contrast, the freshwater environment has highly variable concentrations of both HCO_3^- and CO_2 and CO_2 from the sediment or atmosphere can be accessed, so some species would not benefit from using HCO_3^- however, a highly effective CCM is needed to continue to photosynthesise during episodes of extreme carbon depletion. Below, three examples of the interaction between CCMs and ecology are outlined.

CCMs and distribution of freshwater macrophytes

The high CO_2 concentrations that exist in some freshwater environments allow plants that grow there to survive without a CCM. For example, the freshwater moss *F. antipyretica* lacks a CCM but survives in a lake, Esthwaite Water UK, where surface concentrations of CO_2 in summer are extremely low (Fig. 2). This is possible because it grows close to the sediment surface where CO_2 concentrations were on average about 120 mmol m^{-3} and reached a maximum of 270 mmol m^{-3} and were close to saturating for *F. antipyretica* (Maberly, 1985a, b). In another example, *Fontinalis antipyretica* and the submerged form of *Berula erecta*, both of which are restricted to CO_2, grew immediately downstream of the source of the River La Sorgue, in southern France, fed by groundwater where the CO_2 concentration was in excess of 400 mmol m^{-3} (Maberly *et al.*, 2015). Species of low stature may benefit from the locally high concentrations of CO_2 above the sediment surface and these may account for at least 15% of the over 100 species tested (Fig. 5) but this is almost certainly an underestimate of the importance of this strategy since seedlings or small shoots will also benefit from this carbon sources as will species growing in otherwise heterotrophic areas with generally elevated CO_2 concentrations. Carnivorous plants such as *Aldrovanda vesiculosa* and species of *Utricularia* that typically grow in dystrophic sites with low nutrient concentrations, low oxygen concentrations but high CO_2 concentrations also lack a CCM (Adamec, 1997a, b, 2009). However, one population of *U. australis* from a site with low CO_2 showed some evidence for HCO_3^- use (Adamec, 2009) so more work is needed on the extent of genotypic or phenotypic plasticity in this species. Rivers tend to have higher concentrations of CO_2 than lakes because they are closer to the source of CO_2 produced by decomposition processes within the catchment (Sand-Jensen and Frost-Christensen, 1998). Comparing species of freshwater macrophyte found in both rivers and lakes using the pH-drift technique, those from rivers had a slightly lower median ability to extract inorganic carbon than those in lakes (Baattrup-Pedersen *et al.*, 2013).

The link between water chemistry and freshwater macrophyte distribution has been known for many decades (e.g. (Iversen, 1929; Hutchinson, 1970)), and Hutchinson (1970) made this link explicitly for species of *Myriophyllum*. These patterns, particularly since pH and alkalinity are often the key variables linked to distribution, have been associated with macrophyte carbon-physiology e.g. (Spence, 1967) and . An example can be seen for the survey of macrophytes in 82 Danish lakes, reported by (Vestergaard and Sand-Jensen, 2000). The lakes were categorised into five groups based on their macrophyte communities and the concentrations of HCO_3^- and CO_2 in the five lake groups were measured. Here, the macrophytes within each of these groups were assigned to whether or not they could use HCO_3^- and the extent to which they could deplete inorganic carbon based on pH-drift experiments using the information compiled in Supplementary Table 1. The average difference in the ability of the macrophytes in each group to exploit the inorganic carbon reserves was significantly related to the carbonate chemistry: the percent carbon available increased with HCO_3^- concentration and pH and decreased with CO_2 (Fig. 6). Very similar results were obtained based on the proportion of species able to use HCO_3^- instead of ability to extract inorganic carbon (data not shown). While numerous environmental conditions control the distribution of macrophytes, the results in Fig. 6 show that high concentrations of HCO_3^- and low concentrations of CO_2 favour species with an ability to use HCO_3^-. These two factors are linked since HCO_3^- concentration (alkalinity) along with lake depth can be used to predict background phosphorus concentration in lakes (Vighi and Chiaudani, 1985) and this nutrient often limits lake productivity, and hence controls summer depletion of CO_2.

CCMs and distribution of freshwater phytoplankton

Within freshwater phytoplankton, species from acid sites where concentrations of HCO_3^- are minimal or absent have a lower ability to remove inorganic carbon than species from neutral or alkaline sites. The ellipsoidal form of the trebouxiophyte *Watanabea* sp., which in culture at pH 2 was the predominant form at low CO_2 concentrations (Diaz and Maberly, 2009), appeared to operate a CCM but in the absence of HCO_3^- at this pH, this must have been based on active uptake of CO_2. The spheroidal form of this species and other species tested from the highly acid Lake Caviahue and its inflows in Argentina appeared to largely lack CCMs. Similarly, the acidophile *Chlamydomonas acidophila* and the acidotolerant *C. pitschmannii*, with optima for growth of pH 3.6 and pH 5.3 respectively, mainly relied on CO_2 as a source of inorganic carbon (Lachmann *et al.*, 2016). In contrast, the neutrophiles *C. reinhardtii* and *Scenedesmus vacuolatus* with optima for growth of pH 6.3 and 8.1 respectively, were effective HCO_{3-} users. A similar link between pH preference and the presence or effectiveness of a CCM has also been shown for desmids. Species such as *Staurastrum chaetoceras* and *S. planktonicum* found in alkaline lakes were highly effective at removing inorganic carbon, while species such as *S. brachiatum* restricted to acidic water had a much less effective CCM (Spijkerman *et al.*, 2005).

To illustrate the spatial and temporal variation in carbon removal ability in freshwater phytoplankton, samples were collected in spring and summer from four lakes in Cumbria which differed in their alkalinity, productivity and extent of carbon depletion (Fig. 7). Net phytoplankton from each lake were assessed for their ability to remove inorganic carbon using pH-drift experiments. There were large differences among seasons and lakes in the ability of the phytoplankton to remove inorganic carbon, but adjusting the concentration of HCO_3^- to a standard value had no significant effect (ANOVA, $P = 0.000$ for lake and season but $P = 0.089$ for bicarbonate treatment). In the least productive lake, Derwentwater, where surface concentrations of CO_2 never fell below air-equilibrium, minimum CO_2 concentrations at the end of the drift were greater than 1 mmol m^{-3} in spring and summer suggesting that the phytoplankton population as a whole did not have a CCM. In contrast, in the most productive lake, Esthwaite Water, where summer lake CO_2 concentrations fell to around 100-times below air-equilibrium (~0.17 mmol m^{-3}), the final CO_2 in drifts were substantially below the nominal 1 mmol m^{-3} threshold in spring and summer. The difference between the lake phytoplankton in their ability to remove inorganic carbon was closely related to species composition. In the lakes where low CO_2 concentrations favoured cyanobacteria (Shapiro, 1997) the phytoplankton population had a much greater ability to remove carbon than in those dominated by diatoms and chrysophytes (Fig. 7E,F). Thus, the ability of the phytoplankton population to remove inorganic carbon decreased with increasing % biovolume of diatoms and chrysophytes and conversely increased with increasing % biovolume of cyanobacteria. This is consistent with seasonal difference seen in Esthwaite Water with spring diatoms having a lower ability to remove inorganic carbon than the summer cyanobacteria (Talling, 1976). These results suggest that inorganic carbon removal from productive lakes shifts the phytoplankton population towards species with effective CCMs, although the dynamic nature of these lakes where wind mixing can entrain pH from depth (Maberly, 1996) means that short-lived windows of higher CO_2 may occur that could be exploited by rapidly-growing species without a CCM.

There is also evidence for intraspecific carbon competition within the widespread cyanobacterium, *Microcystis* that can dominate during high-pH episodes in nutrient enriched lakes (Talling, 1976). A study of 20 different strains of *Microcystis aeruginosa* showed variation in the presence of genes coding for different components of the CCM (Sandrini *et al.*, 2014). Thus while all 20 strains possessed genes for the HCO_3^- uptake system *BCT1*, eleven strains lacked the HCO_3^- transporter gene *BicA* and another strain lacked the HCO_3^- transporter gene *SbtA*. Measurements in a lake, and in laboratory experiments, showed that there was a trade-off between the activity of these transporters and inorganic carbon availability. When inorganic carbon availability was high, populations were dominated by genotypes with the *bicA* gene which had a low affinity for HCO_3^- but a high rate of flux, while at low inorganic carbon availability populations were dominated by genotypes with *SbtA* which has a much higher affinity for HCO_3^- but a lower rate of flux (Sandrini *et al.*, 2016). Thus, the effectiveness of cyanobacterial CCMs, the high phenotypic variability of a given genotype and physiological differences among genotypes make cyanobacteria powerful competitors for inorganic carbon with other types of phytoplankton.

Growth in low light environments

Unlike the other marine photosynthetic organisms, marine macroalgae had a large range of ability to exploit the reserves of inorganic carbon. In agreement with expectations related to the additional energy costs of operating a CCM, there are indications that marine macroalgae growing in subtidal or shaded environments lack or have a low CCM activity. Thus in a survey of 35 marine macroalgae, species from the sublittoral, especially understory species that might be further shaded by larger kelps, had a significantly lower ability to remove inorganic carbon than intertidal or rockpool species and largely lacked a CCM (Maberly, 1990). Many of these shaded subtidal species were Rhodophyta and in a study restricted to this group, Murru and Sandgren (2004) found a similar pattern and a larger synthesis of data for all types of marine macroalgae reinforced this (Stepien, 2015; Stepien *et al.*, 2016). Thus the cost of operating a CCM in a low-light environment appears to outweigh the benefits which are arguably low because light, rather than inorganic carbon, may be limiting and concentrations of CO_2 unlikely to be depleted below air equilibrium.

Low light usually restricts the growth of freshwater macrophytes at depth (Spence, 1967). A compilation of depth limits and minimum light requirements for different groups of freshwater macrophytes showed that bryophytes were often found at the depth limit and had the lowest light requirement of about 2.2% of surface light (Middelboe and Markager, 1997) while depth limits for charophytes, elodeid and isoetid macrophytes were at higher light levels. Since virtually all freshwater bryophytes lack a CCM, the saved energy costs may be one of the characteristics permitting growth at low light.

Conclusions

Dissolved inorganic carbon is potentially more limiting for aquatic photoautotrophs than CO_2 in air is for terrestrial plants because of low rates of CO_2 diffusion in water and, particularly in fresh waters, strong seasonal depletion of inorganic carbon. CCMs are consequently more important in aquatic compared to terrestrial systems both in terms of species numbers and contribution to productivity. There is a large range of CCM mechanisms and carbon-extraction capabilities in aquatic photoautotrophs, particularly in fresh waters where the range of concentrations of CO_2 and HCO_3^- is large. Marine photoautotrophs are more similar to one another in terms of ability to remove carbon and generally better able to extract carbon, probably because the benefit of using HCO_3^- is greater given its high concentration and because alternative reserves of CO_2 in the sediment or atmosphere are largely unavailable, unlike for freshwater macrophytes. The variation in inorganic concentration and variability in CCM capability results in inorganic carbon being an ecological factor that controls ecological distribution. At low light, because of the energy cost associated with operating a CCM, and the lower benefits of increasing rates of photosynthesis, photoautotrophs from these environments often lack or have a low capacity to operate a CCM. Future work should focus on exploring the diversity of CCMs in the understudied seagrasses and the numerous phylogenetic groups of marine phytoplankton and exploit the biochemical and molecular tools and approaches that have been developed for microalgae to generate a mechanistic understanding of CCM mechanisms in larger aquatic photoautotrophs. Strengthening the links between ecology and CCMs will increase our understanding of the mechanisms underlying ecological success, aquatic productivity and species distribution and will place mechanistic studies in a clearer ecological context.

Supplementary data

Supplementary methods associated with figures 1 to 7; Supplementary Table 1. Ability of different species of aquatic photoautotroph to remove inorganic carbon (C_T) during pH-drift experiments.

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Table 1. Annual average concentrations of CO_2 from a global dataset of lakes and rivers. CO_2 data derive from (Raymond *et al.*, 2013) Supplementary Information and the alkalinity data derive from the GLORICH database (Hartmann *et al.*, 2014).

Environment	25 percentile	50 percentile	75 percentile
Rivers CO_2 (µatm)	964	1598	2311
Lakes CO_2 (µatm)	340	736	915
Rivers CO_2 (mmolm^{-3})*	44	73	105
Lakes CO_2 (mmolm^{-3})*	16	34	42
Rivers alkalinity (mequiv m^{-3})	540	1559	3056

*Calculated from µatm for an assumed temperature of 15°C.

Figure legends

Fig. 1. Inorganic carbon chemistry in aquatic systems. A, solubility of CO_2 (solid lines) and O_2 (dashed lines) in fresh water (orange) and sea water (blue) as a function of temperature. B, effect of ionic strength on pK1' (solid lines) and pK_2' (dashed lines) at 5°C (blue), 15°C (grey) and 25°C (orange), seawater values shown by circles. C, pH at equilibrium with water at different alkalinities at 280 ppm (blue) 400 ppm (grey) and 560 ppm (orange); seawater values shown as circles. The black line shows pK_1' for fresh water. D, Bjerrum plot of CO_2 (orange), HCO_3^- (grey) and CO_3^{2-} (blue) for seawater (solid line) and freshwater with alkalinities representing the 25th (dotted line) and 75th percentile (dashed line) in Table 1. More detail is provided in Supplementary methods.

Fig. 2. Seasonal changes in carbonate chemistry in a productive lake, Esthwaite Water UK, during 1993. A, pH; B, concentration of CO_2; C, concentration of HCO_3^-; and D, concentration of CO_3^{2-}. Hourly values are shown (blue line) in comparison to values calculated for equilibrium with an atmosphere containing 360 ppm CO_2 (orange line). The insets show the ranked values; note [CO_2] is on a log scale, with the mean air-equilibrium concentration shown by a circle. More detail is provided in Supplementary methods.

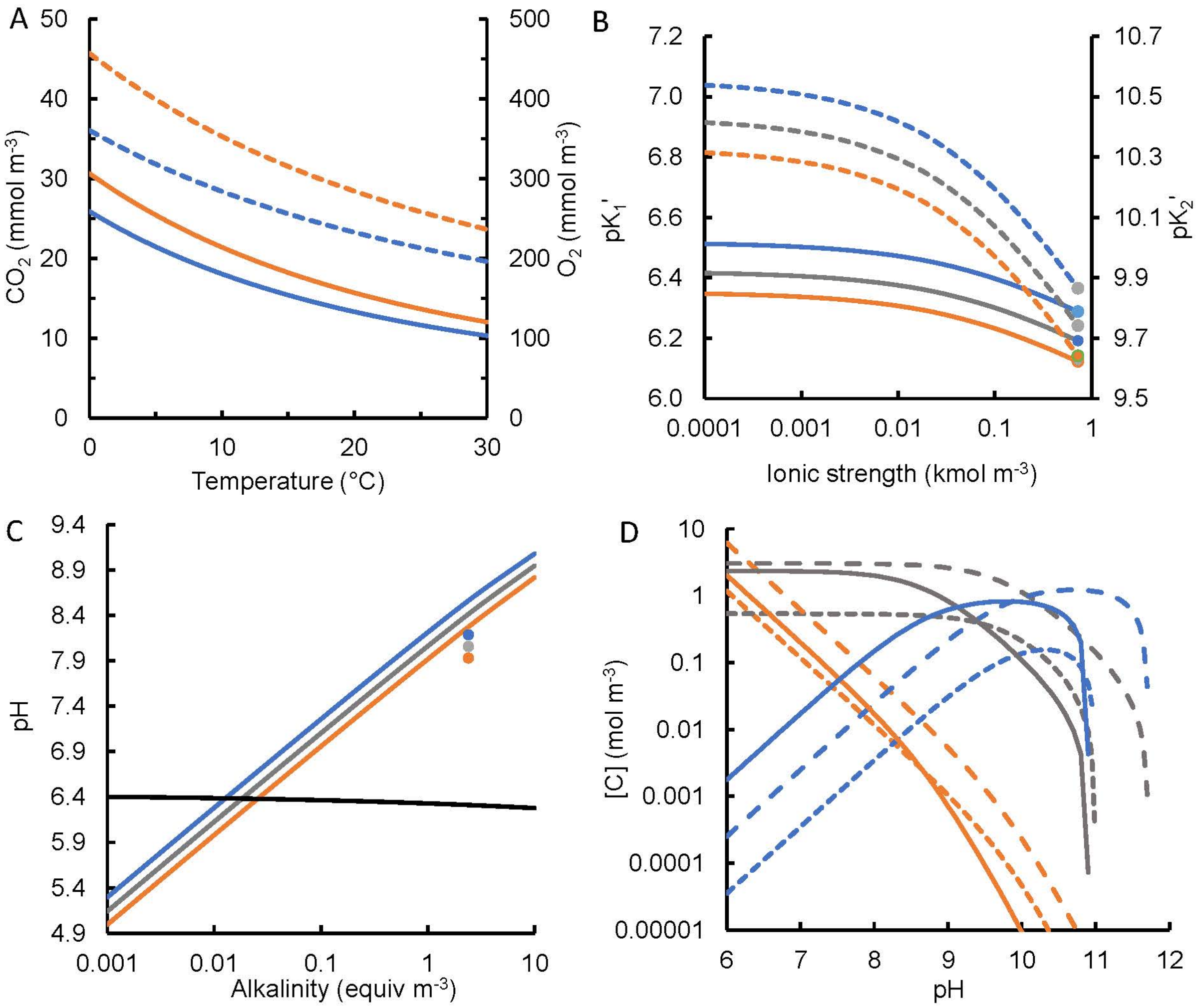
Fig. 3. Comparison of rate of carbon uptake vs concentration of total inorganic carbon during a pH-drift experiment for three species of *Myriophyllum*. Rates are expressed as a function of concentration of: A, C_T and B, CO_2. Plants were collected from different sites but grown under standard conditions before the experiments. Drift conditions: alkalinity 1 equiv m^{-3}, temperature 20°C, light 500 µmol m^{-2} s^{-1} photosynthetically available radiation (Maberly unpublished). The vertical line shows the air-equilibrium (400 µatm) conditions. More detail is provided in Supplementary methods.

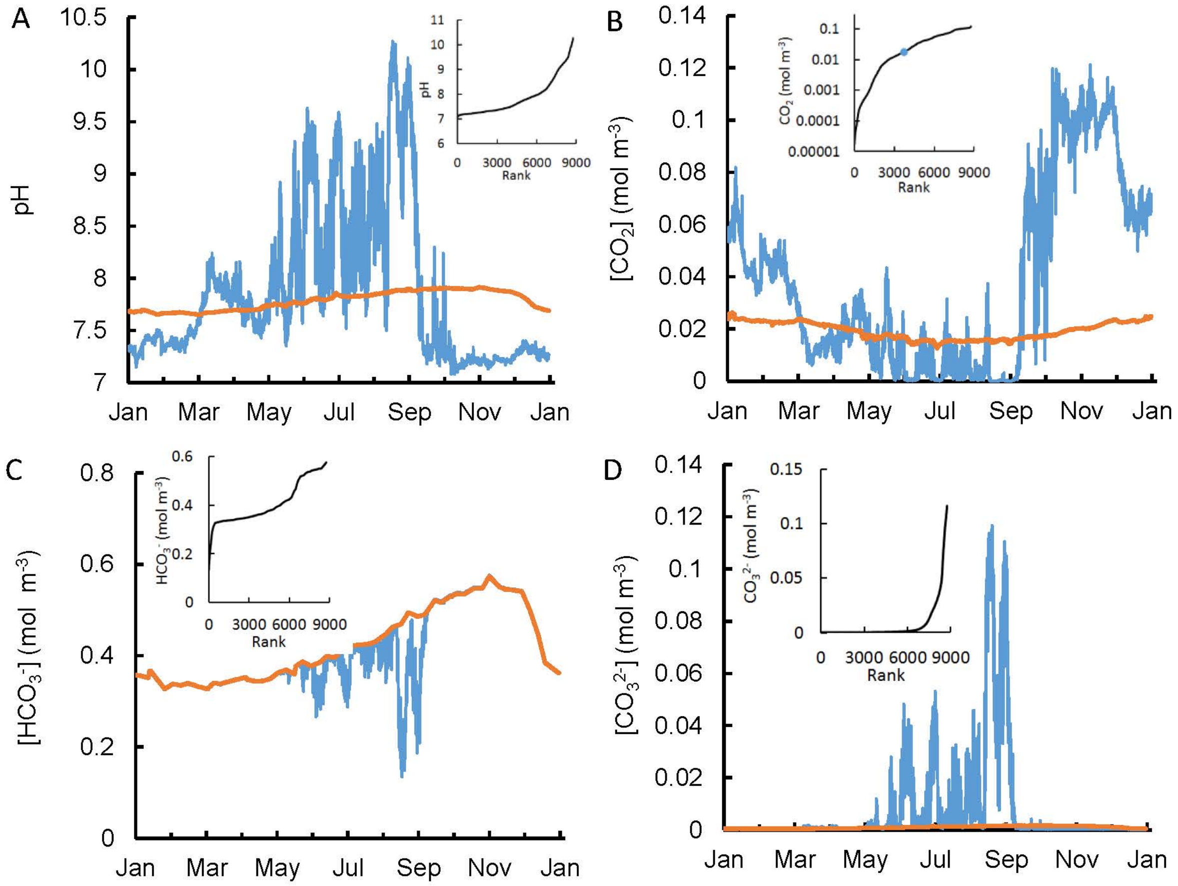
Fig. 4. Gradation in ability to remove inorganic carbon based on pH-drift experiments for different groups of aquatic photoautotrophs. These include in order of decreasing median ability: seagrasses (10 species); marine phytoplankton (13 species); filamentous freshwater algae (34 species); marine macroalgae (142 species); freshwater phytoplankton (37 species), freshwater macrophytes (102 species).The vertical line show the median ability for each group. More detail is provided in Supplementary methods and the data are available in Supplementary Table 1.

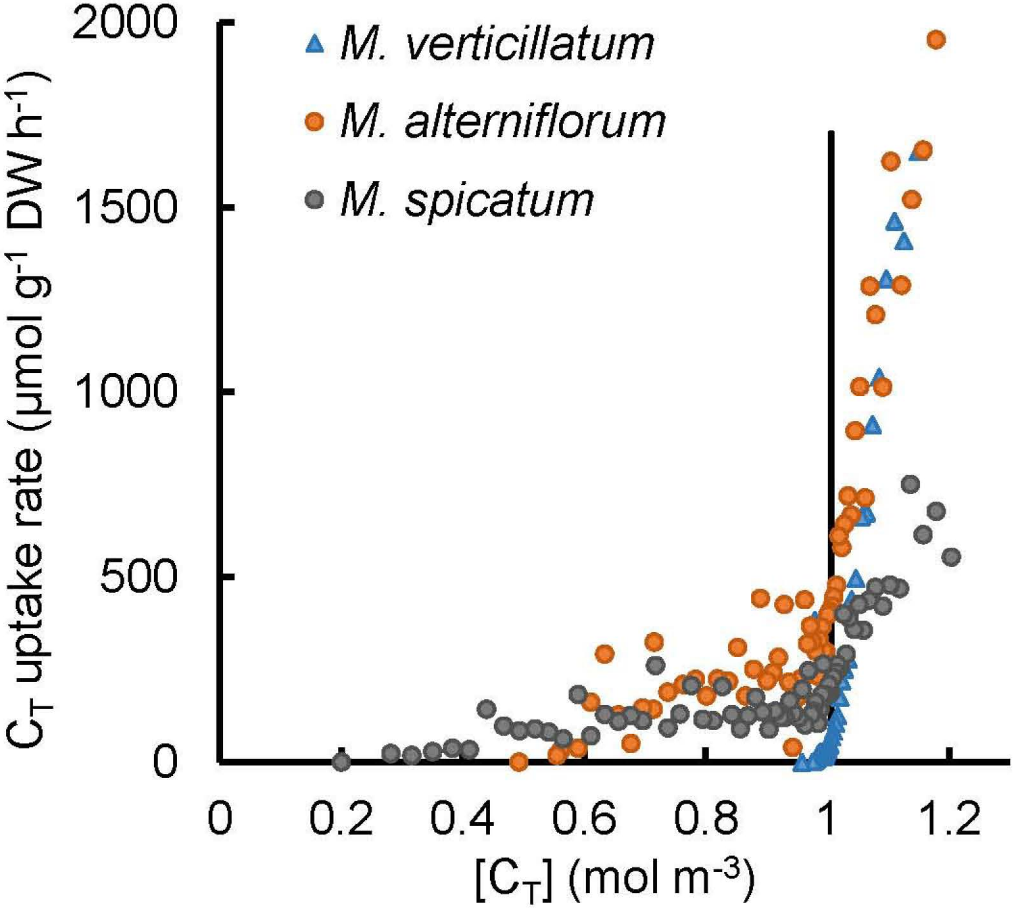
Fig. 5. Number of species of freshwater macrophyte with different strategies for obtaining inorganic carbon in photosynthesis. A, species, categorised following (Klavsen *et al.*, 2011); B, Percentage of CO_2 only or HCO_3^- species with alternative strategies. More detail is provided in Supplementary methods.

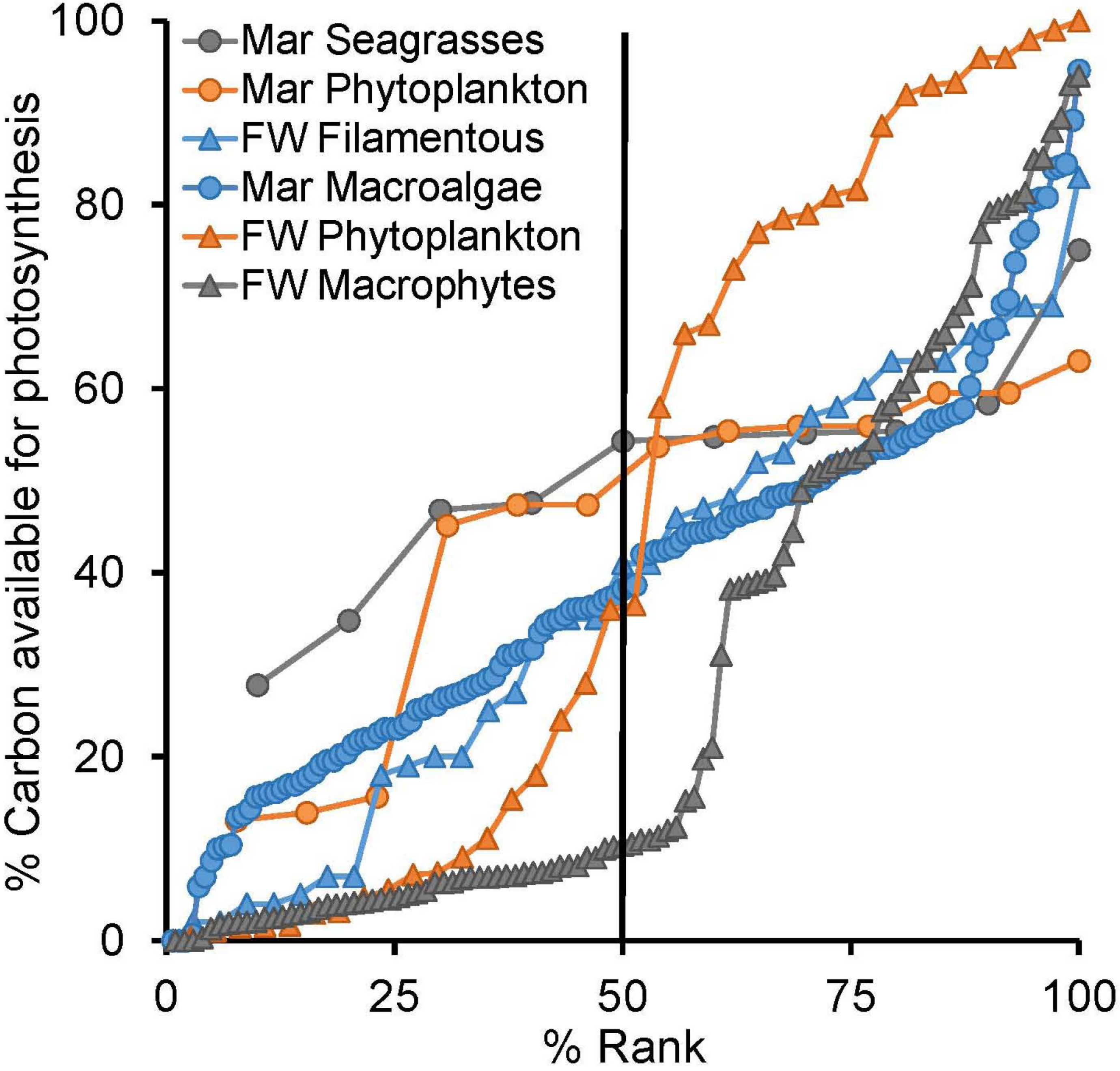
Fig. 6. Relationship between the ability of submerged macrophytes to remove inorganic carbon and their presence in groups of Danish lakes of different water chemistry. Lake groups characterised in terms of A, alkalinity, B, CO_2 concentration and C, pH. Error bars show one standard deviation; the group means were fitted to a log or linear equation the parameters of which are shown. Lake data derived from (Vestergaard and Sand-Jensen, 2000). More detail is provided in Supplementary methods.

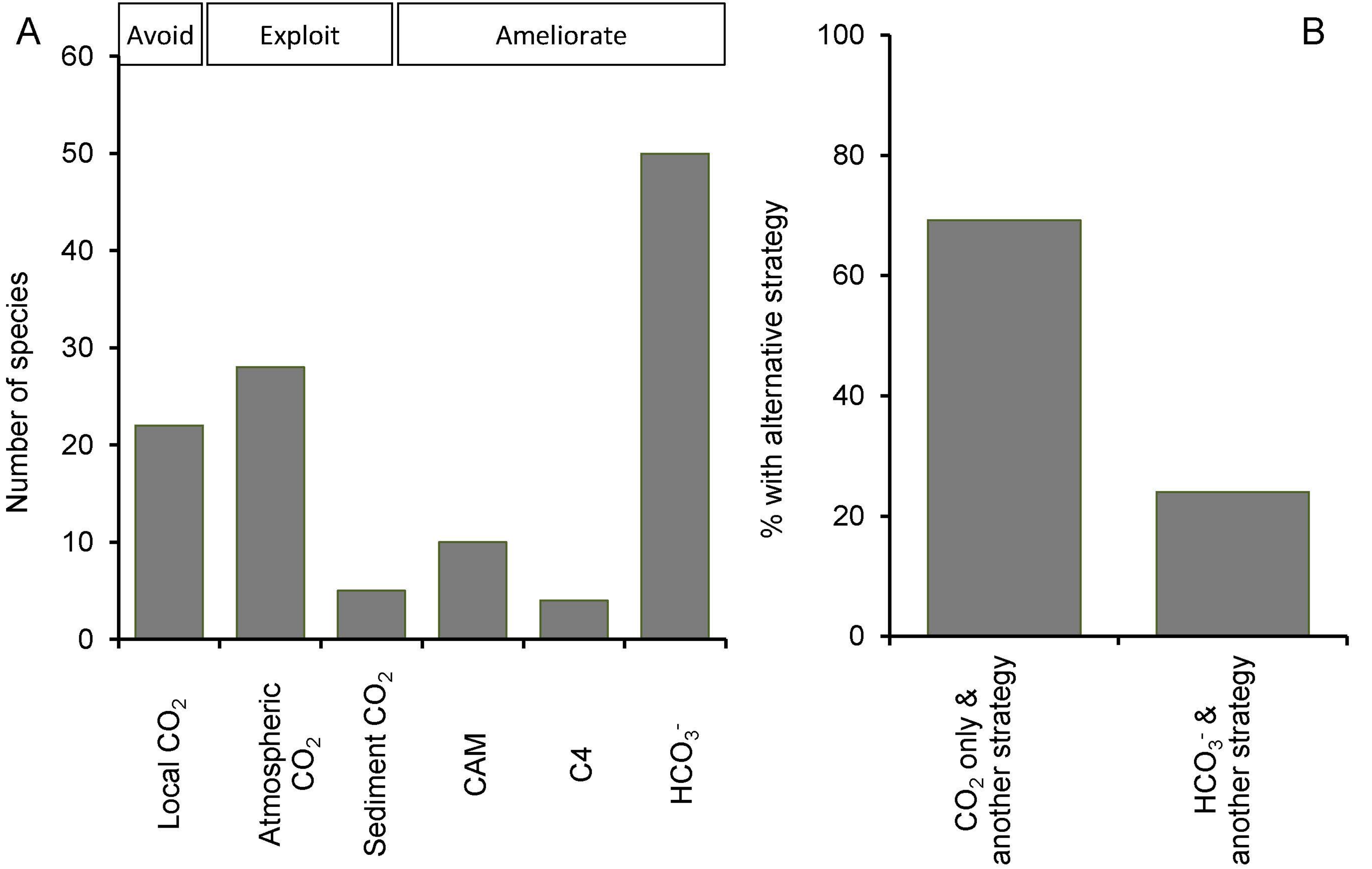
Fig. 7. Seasonal and phylogenetic variation in ability of freshwater phytoplankton to remove inorganic carbon. A, seasonal variation in CO_2 concentration (log scale) for Derwentwater (blue, Ullswater (orange), Windermere South Basin (grey) and Esthwaite Water (Green), the blue shading shows times when the experiments were performed; B, as for panel A, but HCO_3^- concentration; C, % carbon removal for the four lake in spring at ambient (grey) or a standard (blue) concentration of HCO_3^- of about 0.55 mmol m^{-3}, error bars show one standard deviation; D, as for panel C but for summer; E, % carbon available as a function of % contribution of diatoms plus chrysophytes to total biovolume in spring (blue) or summer (orange) with polynomial fit for combined seasonal data; F, as for panel E, but % contribution of cyanobacteria. More detail is provided in Supplementary methods

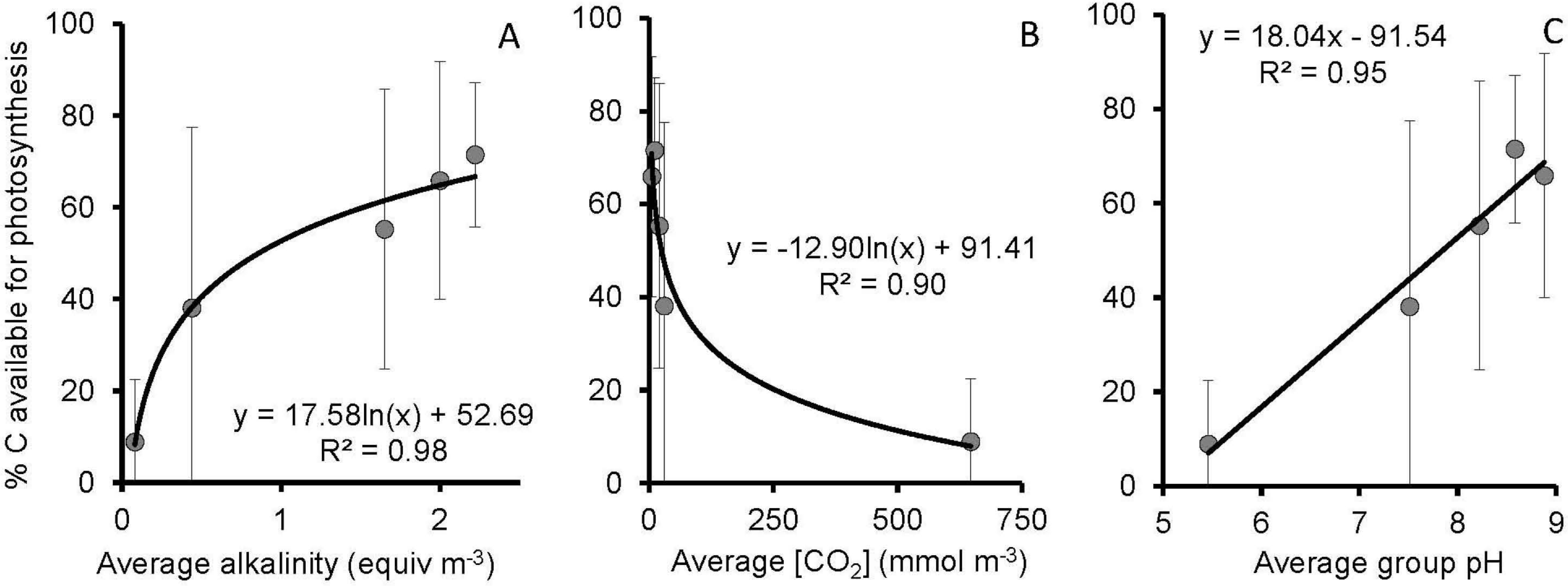

A
CO$_2$ (mmol m^{-3})
O$_2$ (mmol m^{-3})
0
10
20
30
40
50
0
100
200
300
400
500
Temperature (°C)
B
pK$_1$'
pK$_2$'
6.0
6.2
6.4
6.6
6.8
7.0
7.2
9.5
9.7
9.9
10.1
10.3
10.5
10.7
0.0001
0.001
0.01
0.1
1
Ionic strength (kmol m^{-3})
C
pH
4.9
5.4
5.9
6.4
6.9
7.4
7.9
8.4
8.9
9.4
0.001
0.01
0.1
1
10
Alkalinity (equiv m^{-3})
D
[C] (mol m^{-3})
0.00001
0.0001
0.001
0.01
0.1
1
10
6
7
8
9
10
11
12
pH


A
10.5
10
9.5
9
8.5
8
7.5
7
pH
Jan
Mar
May
Jul
Sep
Nov
Jan
11
10
9
8
7
6
pH
0
3000
6000
9000
Rank
B
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
$[CO_2]$ (mol m^{-3})
Jan
Mar
May
Jul
Sep
Nov
Jan
0.1
0.01
0.001
0.0001
0.00001
CO_2 (mol m^{-3})
0
3000
6000
9000
Rank
C
0.8
0.6
0.4
0.2
0
$[HCO_3^-]$ (mol m^{-3})
Jan
Mar
May
Jul
Sep
Nov
Jan
0.6
0.4
0.2
0
HCO_3^- (mol m^{-3})
0
3000
6000
9000
Rank
D
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
$[CO_3^{2-}]$ (mol m^{-3})
Jan
Mar
May
Jul
Sep
Nov
Jan
0.15
0.1
0.05
0
CO_3^{2-} (mol m^{-3})
0
3000
6000
9000
Rank


M. verticillatum
M. alterniflorum
M. spicatum
2000
1500
1000
500
0
C_T uptake rate (µmol g^{-1} DW h^{-1})
0
0.2
0.4
0.6
0.8
1
1.2
$[C_T]$ (mol m^{-3})


Mar Seagrasses
Mar Phytoplankton
FW Filamentous
Mar Macroalgae
FW Phytoplankton
FW Macrophytes
% Carbon available for photosynthesis
100
80
60
40
20
0
0
25
50
75
100
% Rank


A
Avoid
Exploit
Ameliorate
Number of species
0
10
20
30
40
50
60
Local CO_2
Atmospheric CO_2
Sediment CO_2
CAM
C4
HCO_3^-
B
% with alternative strategy
0
20
40
60
80
100
CO_2 only & another strategy
HCO_3^- & another strategy

% C available for photosynthesis
100
80
60
40
20
0
A
y = 17.58ln(x) + 52.69
R² = 0.98
0
1
2
Average alkalinity (equiv m-3)
100
80
60
40
20
0
B
y = -12.90ln(x) + 91.41
R² = 0.90
0
250
500
750
Average [CO2] (mmol m-3)
100
80
60
40
20
0
C
y = 18.04x - 91.54
R² = 0.95
5
6
7
8
9
Average group pH