The differential ability of two species of seagrass to use carbon dioxide and bicarbonate and their modelled response to rising concentrations of inorganic carbon

Stephen Christopher Maberly¹, Andrew W. Stott¹ and Brigitte Gontero²*

¹UK Centre for Ecology & Hydrology, Lancaster Environment Centre, Lancaster, United Kingdom, ²Aix Marseille Univ, CNRS, BIP, UMR 7281, IMM, Marseille, France

Seagrass meadows are one of the most productive ecosystems on the planet, but their photosynthesis rate may be limited by carbon dioxide but mitigated by exploiting the high concentration of bicarbonate in the ocean using different active processes. Seagrasses are declining worldwide at an accelerating rate because of numerous anthropogenic pressures. However, rising ocean concentrations of dissolved inorganic carbon, caused by increases in atmospheric carbon dioxide, may benefit seagrass photosynthesis. Here we compare the ability of two seagrass from the Mediterranean Sea, *Posidonia oceanica* (L.) Delile and *Zostera marina* L., to use carbon dioxide and bicarbonate at light saturation, and model how increasing concentrations of inorganic carbon affect their photosynthesis rate. pH-drift measurements confirmed that both species were able to use bicarbonate in addition to carbon dioxide, but that *Z. marina* was more effective than *P. oceanica*. Kinetic experiments showed that, compared to *Z. marina*, *P. oceanica* had a seven-fold higher affinity for carbon dioxide and a 1.6-fold higher affinity for bicarbonate. However, the maximal rate of bicarbonate uptake in *Z. marina* was 2.1-fold higher than in *P. oceanica*. In equilibrium with 410 ppm carbon dioxide in the atmosphere, the modelled rates of photosynthesis by *Z. marina* were slightly higher than *P. oceanica*, less carbon limited and depended on bicarbonate to a greater extent. This greater reliance by *Z. marina* is consistent with its less depleted $^{13}$C content compared to *P. oceanica*. Modelled photosynthesis suggests that both species would depend on bicarbonate alone at an atmospheric carbon dioxide partial pressure of 280 ppm. *P. oceanica* was projected to benefit more than *Z. marina* with increasing atmospheric carbon dioxide partial pressures, and at the highest carbon dioxide scenario of 1135 ppm, would have higher rates of photosynthesis and be more saturated by inorganic carbon than *Z. marina*. In both species, the proportional
Introduction

Seagrasses are marine angiosperms from four families (or five if the brackish water Ruppiaceae are included), all from the monocot order Alismatales, that evolved ultimately from land plants, probably via freshwater ancestors, and first returned to the sea in the Cretaceous period, around 140 to 100 million years ago (Les et al., 1997; Wissler et al., 2011; Les and Tippery, 2013). Seagrass meadows deliver many globally-important and locally-important ecosystem goods and services (Larkum et al., 2006). Globally, they cover an area of between 0.15 to 4.32 \times 10^6 \text{km}^2 and sustain a variety of fish, crustaceans, and invertebrates (Long et al., 2020) and gas phase oxygen concentrations in internal lacunae may be even higher. These diurnal changes have the potential to stimulate photorepiration and limit photosynthesis to an even greater extent than under atmospheric equilibrium conditions. Interestingly, Rubisco from seagrasses had a lower catalytic efficiency (maximum catalytic rate divided by \(K_M\)) for oxygen than terrestrial plants or freshwater plants (Capó-Baucà et al., 2022) which might be an adaptation to reduce photorepiration and perhaps represents a trade-off with their higher \(K_M\) for CO₂.

In response to inorganic carbon limitation, many aquatic plants have evolved a range of carbon acquisition strategies (Klavsen et al., 2011) some of which are based on active CO₂ concentrating mechanisms (CCMs) (Larkum et al., 2017; Maberly and Gontero, 2017; Maberly and Gontero, 2022). Many, but not all, species of seagrasses, have CCMs that exploit the high concentration of bicarbonate (HCO₃⁻ \(-2\) mmol L⁻¹) in the ocean (Beer et al., 2002; Larkum et al., 2006; Koch et al., 2013; Borum et al., 2016; Larkum et al., 2017). Since seagrass cells have a membrane potential of about -160 to -170 mV [i.e. negative inside the cell; (Rubio and Fernández, 2019)] and the plasmalemma is relatively impermeable to ions, HCO₃⁻ cannot enter the leaf passively and HCO₃⁻ use has to be an active process (Raven, 1970). In some freshwater plants, in addition to HCO₃⁻ use, biochemical CCMs involving C₄ photosynthesis and Crassulacean Acid Metabolism are found (Maberly and Gontero, 2018), however, there is little evidence that they are important in seagrasses (Koch et al. 2013; Larkum et al., 2017). Carbonic anhydrase catalyzes the interconversion of CO₂ and HCO₃⁻ and is present ubiquitously (Supuran, 2018; Jensen et al., 2020) and is involved in CCMs. An external, periplasmic, carbonic anhydrase, between the plasmalemma and cell wall, is frequently involved in facilitating inorganic carbon uptake (James and Larkum, 1996; Larsson and Axelsson, 1999;
Moroney et al., 2011; Tachibana et al., 2011; Van Hille et al., 2014; Fernandez et al., 2018). Solute carrier proteins from family 4 (SLC4) are involved in anion exchange (Romero et al., 2013) and have been implicated in facilitating HCO$_3^-$ uptake in a range of aquatic species (Drechsler et al., 1993; Bjork et al., 1997; Nakajima et al., 2013; Fernandez et al., 2014; Poliner et al., 2015; Huang et al., 2020).

Seagrasses are threatened worldwide by direct and indirect human interference that includes physical disturbance, pollution and nutrient enrichment, increased sediment load and climate change (Orth et al., 2006; Waycott et al., 2009). Globally, seagrass meadows have been estimated to be declining by 110 km$^2$ y$^{-1}$ since 1980 and the rate of loss is accelerating (Waycott et al., 2009). Increasing CO$_2$ in the atmosphere is causing oceanic surface water temperature to increase (IPCC, 2021). It also causes the concentrations of CO$_2$ and HCO$_3^-$ to increase, while pH is declining (Raven et al., 2005; IPCC, 2021). Ocean acidification can be harmful, especially to those species that produce calcite exoskeletons as it causes the concentration of carbonate to decline (Doney et al., 2009). However, the increasing concentration of CO$_2$ has the potential to favor seagrass photosynthesis by reducing carbon limitation (Hall-Spencer et al., 2008; Burnett et al., 2014; Garrard and Beaumont, 2014; Pajusalu et al., 2016). Borum et al. (Borum et al., 2016) found differences among nine Australian seagrasses in the extent of aquatic species (Drechsler et al., 1993; Bjork et al., 1997; Nakajima et al., 2013; Fernandez et al., 2014; Poliner et al., 2015; Huang et al., 2020).

We studied two species from the Mediterranean Sea. Posidonia oceanica (L.) Delile belongs to the family Posidoniaceae and is endemic to the Mediterranean Sea where it is an important ‘ecological engineer’ (Personnic et al., 2014). Zostera marina L. belongs to the family Zosteraceae and is one of the most intensively studied and widespread species, found in the North Atlantic, North Pacific and Arctic Oceans and also the Mediterranean Sea (Olsen et al., 2014). Both species have been shown to use HCO$_3^-$ as well as CO$_2$ (see references below). The aim of this work was firstly to determine the uptake characteristics of CO$_2$ and HCO$_3^-$ for these two species of seagrass using pH-drift, stable isotope discrimination, measurement of photosynthesis kinetics and analysis of protein sequences. By characterizing the uptake kinetics of HCO$_3^-$ from CO$_2$, separately, the second aim was to evaluate the response of the two species to rising CO$_2$ using a model based on the kinetic measurements and scenarios of future increases in atmospheric CO$_2$.

Carbon uptake kinetics

Rates of net photosynthesis were measured as oxygen evolution at 20°C in an electrode chamber (Oxygraph, Hansatech Instruments, Norfolk, UK). The chamber was illuminated with a 35 W halogen GU10C lamp behind a hot-mirror cut-off filter at 750-1100 nm (HMC-1033, UQG Cambridge, UK) to minimize heat input to the chamber. The leaves received ~400 μmol m$^{-2}$ s$^{-1}$ PAR, which preliminary experiments had shown to be saturating, but not photo-inhibiting. Leaves of 25 mm length and 10 mm width (P. oceanica) or 28 mm length and 5 mm width (Z. marina), were rinsed for a few seconds in artificial seawater (Kester et al., 1967) without NaHCO$_3$, boric acid, sodium fluoride or strontium chloride (Supplementary Table 1) and were then incubated for fifteen minutes at 20°C in artificial seawater with 0.2 mM HCO$_3^-$, added as NaHCO$_3$, and bubbled with air. The leaf was placed in the oxygen electrode with 2 mL of the same solution and an initial oxygen concentration of about 70% saturation used. A predetermined volume of 0.1 or 1 mol L$^{-1}$ HC$_3^-$ was then injected into the chamber to lower pH (on the NBS scale) to pHi 7 generating increased concentrations of CO$_2$ at very similar concentrations of HCO$_3^-$ (Supplementary Table 2) and the rate was re-measured. The leaf was then removed from the electrode and incubated for 15 minutes at the next HCO$_3^-$ concentration and rates measured following the same procedure. Five NaHCO$_3$ concentrations were used, up to a maximum of 5 mMmol L$^{-1}$. Each measurement continued until a constant slope was achieved, which typically lasted for around three to six minutes. No additional buffers were used because our preliminary experiments showed that the inclusion of 10 mM HEPES increased the K$\text{½}$ for HCO$_3^-$ 20-fold in P. oceanica and 6-fold in Z. marina. This procedure produced different concentrations of HCO$_3^-$ at air-equilibrium CO$_2$ and higher

Materials and methods

Sampling sites

Two species of seagrass were collected in July 2019 from the Mediterranean Sea near Marseille in the South of France. P. oceanica was collected from St Cyr sur Mer (43° 9.54’N, 5° 37.283’E) at a depth of 18 m. Z. marina was collected from Etang de Thau (43°, 25.1607’N, 3° 36.0107’E) at a depth of 1 m. The salinity was about 38 at both sites and the water temperature was between 21 and 25°C. Plants were transported back to the laboratory in a cool box and kept in an incubator (Innova 4230, New Brunswick Scientific, Edison, New Jersey, USA) at 20°C in natural seawater under continuous illumination from Gro-lux fluorescent tubes producing about 70 μmol m$^{-2}$ s$^{-1}$ photosynthetically active radiation (PAR, 400 to 700 nm; Q201, Macam Photometric, Livingston, UK) and used within two days of collection. Material for the pH-drift and stable carbon isotope analysis was collected two years earlier in July 2017 and used immediately. In 2017, Z. marina was collected from Etang de Thau as in 2019, but in 2017, P. oceanica was collected from the Bay of Marseille (43° 16.38’N, 5° 20.43’E) at a depth of 12 m.

Frontiers in Plant Science
concentrations of CO₂ at near-constant HCO₃⁻ concentration following addition of acid. Concentrations of CO₂ varied between 0.013 and 0.31 mM and HCO₃⁻ concentrations varied between 0.20 and 3.93 mM (Supplementary Table 2). The measurements were made in triplicate.

At the end of the sequence, the leaves were blotted quickly and weighed to determine the fresh weight. The leaves were photographed and leaf area estimated using AreaAna software (Huazhong University of Sciences and Technology, China). A subsample was ground in a mortar and pestle and the pigments extracted in 100% ethanol in the dark overnight at 4°C. Following centrifugation and measurement of optical density a range of atmospheric CO₂ partial pressures

Estimated rates of photosynthesis under a range of atmospheric CO₂ partial pressures

The kinetic data were used to forecast rates of photosynthesis, at 20°C and light saturation, for variable concentrations of CO₂ at a constant seawater carbonate alkalinity of 2.29 mequiv L⁻¹ and a salinity of 36. CO₂-dependent rates were estimated for CO₂ concentrations from 0.0004 to 0.06 mmol L⁻¹ and HCO₃⁻ dependent rates were estimated for the range of HCO₃⁻ concentrations, 0.63 to 2.26 mmol L⁻¹, that corresponded to the CO₂ concentrations. Rates were extracted at CO₂ partial pressures in the atmosphere of 280 ppm (pre-industrial), 410 ppm (contemporary) and four projections to 2100 based on the shared socioeconomic pathway (SSP) scenarios used in the Sixth Assessment Report by the IPCC (2021; (IPCC, 2021)): 446 ppm for SSP1-2.6, 603 ppm for SSP2-4.5, 867 ppm for SSP3-7.0 and 1135 ppm for SSP5-8.5 (Meinshausen et al., 2020). The calculated pH values and concentrations of CO₂ and HCO₃⁻ at 20°C for the scenarios are given in Supplementary Table 3. The absolute concentration of HCO₃⁻ increases more than CO₂ at equilibrium with increasing atmospheric CO₂ partial pressure but the proportion of HCO₃⁻ to CO₂ decreases. The percent contribution of CO₂-dependent and HCO₃⁻ dependent rates to the sum of both rates (Total V_{max net} ) was calculated.

**pH-drift**

The maximum pH achieved in pH-drift experiments was determined by placing about 0.17 g fresh weight of leaf in 15 mL plastic Falcon tubes with a total volume of 16.3 mL containing 14 mL of natural seawater that had been passed through a 0.45 μm filter. There was consequently a gas headspace of about 2.3 mL. The tubes were capped and placed horizontally in an incubator (Innova 4230, New Brunswick Scientific, Edison, New Jersey, USA) at 20°C under continuous illumination from Grolux fluorescent tubes producing about 70 μmol m⁻² s⁻¹ PAR and shaken at 60 rpm. A combination pH-electrode (Hanna HI 1043, Woonsocket, Rhode Island (USA) was calibrated with buffers at
pH 7 and 10 on the NBS scale and pH was measured after 24 hours and roughly after every 12 hours until a maximum pH had been reached. Four replicates per species were used. A control tube without any leaves was also incubated. Alkalinity was measured at the end of the experiment by Gran titration (Mackereth et al., 1978).

Stable carbon isotope analysis

To determine isotope values of the dissolved inorganic carbon (DIC) source, ten mL of seawater were collected in situ at the two collection sites in evacuated exetainers containing phosphoric acid and stored inverted before analysis (Waldron et al., 2007). In the laboratory, four mL of helium (99.999%) were injected into the headspace of each exetainer to over-pressurize. After shaking and 30 minutes equilibration, a 40 μL gas sample was removed for analysis in an Isoprime Ltd Tracegas Preconcentrator (Isoprime, Manchester, UK) coupled to an Isoprime Ltd isotope ratio mass spectrometer. Pulses of known reference CO₂ and blanks were run prior to each batch.

Material freshly collected from the sea was dried at 80°C and stored in aluminum foil prior to analysis. Small amounts of the plant were re-dried at 105°C, aliquots sealed into 6 x 5 mm tin capsules and loaded into an autosampler (Eurovector Elemental Analyser, Eurovector, Milano Italy) coupled in-line to a stable isotope ratio mass spectrometer. Each sample was combusted at 1020°C with a pulse of oxygen and products were carried by a flow of helium through a reduction reactor containing copper wire at 650°C and dried with magnesium perchlorate. N₂ and CO₂ were separated by a packed GC column and delivered, via an ‘open-split’ to the isotope ratio mass spectrometer. Values were compared to pulses of CO₂ reference gas and to a solid working standard of known isotopic composition. Stable carbon data were expressed in the delta notation (δ¹³C) relative to the Vienna Pee Dee Belemnite (VPDB) standard (Eqn 2). Stable isotope methods for inorganic and organic C were accredited to UKAS ISO17025. The δ¹³C values of potential inorganic carbon sources, CO₂ and HCO₃⁻, were calculated from the measured δ¹³C values of DIC using the temperature-dependent equations of (Mook et al., 1974). Discrimination (Δ) of the leaf δ¹³C values was calculated following (Maberly et al., 1992) (Eqn 3) against CO₂ and HCO₃⁻ and against the proportional amounts of CO₂ and HCO₃⁻ used in ambient conditions that were estimated from the photosynthesis kinetics.

\[
\delta^{13}C = (R_{\text{sample}} - R_{\text{VPDB}}/R_{\text{VPDB}}) \times 1000 \quad \text{(Eqn 2)}
\]

Where \( R_{\text{Sample}} \) and \( R_{\text{VPDB}} \) are the \(^{13}\text{C}:/^{12}\text{C}\) ratios of the sample and standard respectively.

\[
\Delta = (\delta_{\text{source}} - \delta_{\text{plant}})/(1 + \delta_{\text{plant}}) \quad \text{(Eqn 3)}
\]

Inorganic carbon speciation

Inorganic carbon concentrations were calculated using the CO2SYS_calc program in Excel (Pierrot et al., 2006) and converted from molal to molar using a seawater density at 20°C of 1.025 kg L⁻¹ for artificial seawater at a salinity of 36 used in the kinetic experiments and 1.027 for the natural seawater with a salinity of 38 used in the pH-drift experiments and analysis of the stable isotope results.

Sequence analysis

Since the genome of Z. marina has been sequenced (Olsen et al., 2016), we searched for proteins that have been implicated in HCO₃⁻ uptake in other aquatic phototrophs: the anion exchange protein solute carrier type 4 family (SLC4) and carbonic anhydrase in this genome. We performed a BLAST search (https://blast.ncbi.nlm.nih.gov/) with SLC4 isoform 1 from the freshwater plant *Ottelia alismoides* as a query (Huang et al., 2020) and Z. marina as the species. For carbonic anhydrase, we searched (https://www.ncbi.nlm.nih.gov/protein/) with carbonic anhydrase as a term and Z. marina as the species. Location of all the proteins was determined using WoLF PSORT (https://wolfpsort.hgc.jp/). In contrast, the annotated genome of *P. oceanica* is not currently available and so it was impossible to determine the presence or absence of the genes for these proteins.

Statistical analysis

Statistical analyses were carried out in Excel for Microsoft 365.

Results

Ability to deplete CO₂ and HCO₃⁻ in pH-drift experiments

None of the replicates had reached their final pH after 24 hours; four reached the final pH after 44 hours and four after 63 hours. Both species were able to raise the pH of seawater to over 9.5, depleting the CO₂ concentration to 0.041 μmol L⁻¹ (41 x 10⁻⁶ mmol L⁻¹) in the case of *P. oceanica* and 0.010 μmol L⁻¹ (10 x 10⁻⁶ mmol L⁻¹) in the case of *Z. marina* (Table 1). This is lower than could be achieved by passive uptake of CO₂ into the leaf (of the order of 1 μmol L⁻¹) and thus indicates an active process, which is likely to be bicarbonate uptake. *Z. marina* had a greater capacity than *P. oceanica* to raise the pH and deplete the concentration of...
DIC (t-test for difference in final DIC concentration, p<0.05). The final concentration of HCO$_3^-$ was also significantly lower in *Z. marina* than in *P. oceanica* (t-test for difference in final HCO$_3^-$ concentration, p<0.05). The DIC/Alk quotient, representing the proportion of inorganic carbon remaining at the end of the drift compared to alkalinity, was between 0.5 and 0.44 in the two species, much higher than in many freshwater plants that can use HCO$_3^-$ because of the higher concentration of unavailable carbonate ions in seawater compared to fresh water.

Rates of photosynthesis as a function of CO$_2$ and HCO$_3^-$

The rates of photosynthesis by *P. oceanica* and *Z. marina* increased with the concentration of DIC and were strongly stimulated when the pH was decreased to pH 7, generating CO$_2$ (Figure 1), particularly for *P. oceanica*. Rates can be normalized to leaf area, chlorophyll $a$ or chlorophyll $a$ and $b$, and dry weight using the results in Supplementary Table 4. *P. oceanica* had a significantly lower leaf area per g fresh weight and dry weight per g fresh weight than *Z. marina* but a higher chlorophyll content. Results of the modelled kinetic characteristics for CO$_2$ and HCO$_3^-$ uptake are given in Table 2 and the modelled responses visualized in Figure 2. The total uptake rates at inorganic carbon saturation in the two species were not significantly different on a fresh weight basis, however the maximal CO$_2$-dependent rate of photosynthesis in *P. oceanica* was significantly higher than in *Z. marina* and conversely the maximal HCO$_3^-$ dependent rate was significantly higher in *Z. marina* than in *P. oceanica* (Table 2). Values of $K_{50}$ for both CO$_2$ and HCO$_3^-$ were significantly lower in *P. oceanica* than in *Z. marina*. The slope of increasing photosynthesis rate at limiting concentration of CO$_2$, was significantly higher, by a factor of 7, in *P. oceanica* than in *Z. marina*, but the equivalent slope for HCO$_3^-$ was only 1.6-times higher. The slope for HCO$_3^-$ uptake was less than 2% that of CO$_2$ uptake in *P. oceanica* but 8% in *Z. marina* indicating the greater importance of HCO$_3^-$ in the carbon economy of *Z. marina* given that the concentration of HCO$_3^-$ is ~140-fold higher than that of CO$_2$ in seawater.

As atmospheric CO$_2$ partial pressure increases, at equilibrium with the atmosphere, the concentrations of HCO$_3^-$ as well as dissolved CO$_2$ also increase (Supplementary Table 3).

---

**TABLE 1** Concentrations of inorganic carbon at the end of pH drift experiments at 20°C in filtered natural seawater.

|                        | pH     | Alkalinity (mequiv L$^{-1}$) | Alkalinity minus borate (mmol L$^{-1}$) | [DIC] (mmol L$^{-1}$) | [CO$_2$] (mmol L$^{-1}$) | [HCO$_3^-$] (mmol L$^{-1}$) | DIC/Alk |
|------------------------|--------|------------------------------|-----------------------------------------|------------------------|--------------------------|-----------------------------|---------|
| *Posidonia oceanica*   | 9.56 (0.11) | 2.130 (0.113)              | 1.692 (0.121)                           | 0.872 (0.102)          | 41 x 10$^{-6}$ (25 x 10$^{-6}$) | 0.191 (0.061)                | 0.51 (0.03) |
| *Zostera marina*       | 9.83 (0.06) | 1.976 (0.123)              | 1.521 (0.122)                           | 0.671 (0.066)          | 10 x 10$^{-6}$ (3 x 10$^{-6}$) | 0.088 (0.015)                | 0.44 (0.02) |
| Seawater blank         | 7.89   | 2.477                        | 2.374                                   | 2.189                  | 16 x 10$^{-3}$           | 1.965                        | 0.96    |

Molar and molal concentrations were interconverted using a seawater density of 1.027 kg L$^{-1}$. Mean with standard deviation in parentheses, n = 4 apart from the seawater blank that was not replicated. The quotient DIC/Alk is based on the alkalinity minus the borate alkalinity. The mean pH was calculated geometrically.
markedly stimulating rates of net photosynthesis in both species (Figures 3A, B). At equilibrium with 410 ppm, the modelled rate of net photosynthesis in *Z. marina* was about 1.25-times greater than those of *P. oceanica* (Figure 3C). The compensation concentration for CO$_2$ was slightly below the concentration at equilibrium with 410 ppm in both species (Table 2). Nevertheless, CO$_2$ was projected to contribute 26.6% to net photosynthesis under these conditions for *P. oceanica*, but only 7.7% for *Z. marina* (Figure 3D). Overall, rates of photosynthesis by *P. oceanica* were 17% carbon-saturated, while those of *Z. marina* were 28% saturated under ambient conditions (Figure 3E).

### Table 2: Kinetic characteristics of CO$_2$ and HCO$_3^-$ uptake by *P. oceanica* and *Z. marina* determined using the model in equation 1.

| Carbon source | Characteristic | *P. oceanica* | *Z. marina* | P:Z |
|---------------|----------------|---------------|-------------|-----|
| **Both**      | Total $V_{\text{max}}^n$ (μmol O$_2$ g$^{-1}$ FW h$^{-1}$) | 45.5 (7.4) | 35.1 (6.3) | 1.3$^\text{NS}$ |
| CO$_2$        | $V_{\text{max}}^n$ (μmol O$_2$ g$^{-1}$ FW h$^{-1}$) | 38.8 (6.9) | 21.3 (3.8) | 1.8$^*$ |
| CO$_2$        | $K_c$ (mmol L$^{-1}$) | 0.036 (0.007) | 0.100 (0.001) | 0.4$^{**}$ |
| CO$_2$        | Compensation point (mmol L$^{-1}$) | 0.011 (0.001) | 0.010 (0.000) | 1.2$^{**}$ |
| CO$_2$        | Slope (μmol O$_2$ g$^{-1}$ FW h$^{-1}$ mmol$^{-1}$ L$^{-1}$) | 1637 (392) | 234 (42) | 7.0$^*$ |
| HCO$_3^-$     | $V_{\text{max}}^n$ (μmol O$_2$ g$^{-1}$ FW h$^{-1}$) | 6.6 (0.7) | 13.9 (2.5) | 0.5$^*$ |
| HCO$_3^-$     | $K_c$ (mmol L$^{-1}$) | 0.615 (0.123) | 1.175 (0.001) | 0.5$^*$ |
| HCO$_3^-$     | Compensation point (mmol L$^{-1}$) | 0.379 (0.104) | 0.404 (0.001) | 0.9$^{NS}$ |
| HCO$_3^-$     | Slope (μmol O$_2$ g$^{-1}$ FW h$^{-1}$ mmol$^{-1}$ L$^{-1}$) | 28.2 (3.2) | 18.0 (3.3) | 1.6$^*$ |
|               | $R^2$           | 0.992         | 0.989       |       |

Values are the means of three replicates with standard deviation in parentheses. $K_c$ is the modelled $K_c$ plus the compensation concentration and the slope is calculated from the ratio of $V_{\text{max}}^n$ to the modelled $K_c$ concentration. P:Z is the ratio of values for *P. oceanica* and *Z. marina*. $V_{\text{max}}^n$ for CO$_2$ is: $a$ x Total $V_{\text{max}}^n$ and $V_{\text{max}}^n$ for HCO$_3^-$ is: $(1-a)$ x Total $V_{\text{max}}^n$ in Eqn 1. $R^2$ is the mean derived from linear regressions between measured and modelled values for each replicate. The significance of the difference between the two species was determined with a t-test and presented as: NS, not significant, $^*P<0.05$, $^{**}P<0.01$.

**Modelled responses to past and future concentrations of CO$_2$ and HCO$_3^-$**

The kinetic data described above were used to project photosynthesis responses of the two species to the concentrations of CO$_2$ and HCO$_3^-$ for pre-industrial atmospheric CO$_2$ partial pressure (Supplementary Table 3). At the pre-industrial CO$_2$ partial pressure of 280 ppm, the CO$_2$ concentration was below the CO$_2$ compensation point and photosynthesis was solely dependent on HCO$_3^-$ in both species (Figure 3D). Projections were made for a range of
future partial pressures for different shared socioeconomic pathway scenarios (Supplementary Table 3). From an atmospheric partial pressure of CO₂ from 410 to 1135 ppm for the SSP5-8.5 scenario the concentration of CO₂ increased 2.8-fold (by 0.023 mmol L⁻¹) and the concentration of HCO₃⁻ increased 1.1-fold (by 0.245 mmol L⁻¹). With increasing concentration of CO₂, rate of CO₂-dependent photosynthesis increased 9.3-fold and HCO₃⁻ dependent rates increased 1.02-fold in P. oceanica so that CO₂ contributed 77% of net photosynthesis at the highest CO₂ concentration of 1135 ppm. In contrast, rates of CO₂-dependent photosynthesis increased 6.3-fold and HCO₃⁻ dependent rates increased 1.1-fold in Z. marina and CO₂ only contributed 33% of net photosynthesis at the highest CO₂ concentration. Consequently, the increasing CO₂ concentration benefited net photosynthesis of P. oceanica to a much greater extent than Z. marina (Figure 3D). At 410 ppm the rate of Z. marina was 1.25-fold higher than that of P. oceanica but despite an increase in rate of photosynthesis in Z. marina at the highest CO₂ scenario of 1135 ppm for SSP5-8.5, the rate of P. oceanica was 1.76-fold higher than that of Z. marina (Figure 3D). As a result of the high dependence of P. oceanica on CO₂, its carbon saturation increased 3.2-fold from 17% at 410 ppm to 55% at 1135 ppm while carbon saturation of Z. marina increased 1.5-fold from 28% to 41% over the same range (Figure 3E).

Stable carbon isotopes and discrimination against inorganic carbon sources

Plant δ¹³C values were 5.21‰ more depleted in P. oceanica at -14.20‰ than in Z. marina at -8.99‰ (t-test, p<0.01) (Figure 4). The δ¹³C for DIC at the two collection sites were similar at 1.06‰ at the P. oceanica collection site and 0.06‰ at the Z. marina collection site. The calculated values of δ¹³CO₂ at the two sites were -7.86‰ and -8.86‰ and δ¹³HCO₃⁻ were 1.11‰ and 0.11‰ respectively. Discrimination was calculated against CO₂ and HCO₃⁻ individually and also in combination weighted by the estimated contribution of these two carbon sources under ambient conditions using the kinetic model. The discrimination against CO₂ and HCO₃⁻ individually was about 6.3‰ greater in P. oceanica than in Z. marina. The weighted discrimination of P. oceanica at 12.02‰ was 3.72‰ greater than by Z. marina at 8.30‰ (Figure 4).

Analysis of the Zostera marina genome for putative proteins involved in HCO₃⁻ uptake

The Z. marina genome contains two sequences of a protein annotated as a boron transporter (KMZ66170 and KMZ71533).
When blasted against NCBI Non-redundant protein sequences database, the top 99 proteins retrieved had between 80.3 and 74.7% similarity for KMZ66170 and 70.0 and 65.5% similarity for KMZ71533. The top 99 protein sequences were annotated as a boron transporter (63) or a bicarbonate transporter (2) with the remainder unidentified for KMZ66170 and as a boron transporter (74) or a bicarbonate transporter (2) with the remainder unidentified for KMZ71533. Boron transporters and HCO$_3^-$ anion exchange proteins both belong to the SLC4 family (Takano et al., 2002; Thurtle-Schmidt and Stroud, 2016), strongly suggesting that Z. marina possesses SLC4. Using WoLF PSORT, the location of both proteins was predicted to be chloroplastic.

The Z. marina genome contains ten sequences corresponding to carbonic anhydrase (Supplementary Table 4). Of these, two were annotated in NCBI as gamma mitochondrial CA (KMZ56823 and KMZ56166), and a third as chloroplastic (KMZ73424). WoLF PSORT was used to determine the location of all ten sequences: it did not predict the same location for the three sequences where a location is given in NCBI, and both results are presented in Supplementary Table 5. Of particular relevance to carbon acquisition, two proteins were predicted to be extracellular (KMZ64507 and KMZ75401).

Discussion

Use of HCO$_3^-$ in Posidonia oceanica and Zostera marina

The data presented here confirm that both studied species are able to use HCO$_3^-$ since in pH drift experiments, the CO$_2$ concentration was driven from 24-times to 100-times below the concentration consistent with diffusive entry of CO$_2$ of around 1 μmol L$^{-1}$. The maximum pH generated by P. oceanica lies within the range of nine species studied by (Borum et al., 2016) while Z. marina has a slightly greater carbon extraction capacity than the species they studied. The final pH for Phyllospadix scouleri, 9.76 (Stepien et al., 2016) is intermediate between the two species studied here. The final pH-drift values reported here are greater than those in (Capó-Bauçà et al., 2022). The δ$^{13}$C value for P. oceanica reported here at -14.20‰, is less negative than the -16.67‰ reported by (Capó-Bauçà et al., 2022) and more negative than the mean of ten records for this species at -13.5‰ compiled in Appendix S4 of (Stepien, 2015) but within the reported range of -16.20 to -12.1‰. Our value for Z. marina of -8.99‰ is less negative than the value of -12.28‰ of Capó-Bauçà et al. (2022) and the mean of 27 values for this species (Appendix S4 of (Stepien, 2015)) but within their measured range of -13.63 to -8.34‰. Z. marina appears to be consistently less depleted in $^{13}$C than P. oceanica. This is in agreement with the physiological measurements reported here, suggesting that Z. marina has a greater dependence on HCO$_3^-$ under ambient conditions than P. oceanica. The δ$^{13}$C values for Z. marina are too positive for Z. marina are too large to be explained by CO$_2$ use alone, while those of P. oceanica are only just explicable if the use of CO$_2$ is very strongly limited by diffusion (Maberly et al., 1992; Raven et al., 2002).

Buffers can substantially increase the $K_{\text{HCO}_3}$ concentration of HCO$_3^-$ uptake in seagrasses (Hellblom et al., 2001): all $K_{\text{HCO}_3}$ concentrations given here were thus measured in the absence of buffers. At a background CO$_2$ concentration of 15 to 19 μmol L$^{-1}$ Invers et al. (2001) reported $K_{\text{HCO}_3}$ concentrations for HCO$_3^-$ of 0.44 mmol L$^{-1}$ for P. oceanica and Rubio et al. (2017) found a $K_{\text{HCO}_3}$ for HCO$_3^-$ uptake of 0.35 mmol L$^{-1}$ which is lower than we reported here for this species at 0.62 mmol L$^{-1}$ (Table 2). The $K_{\text{HCO}_3}$ values for HCO$_3^-$ for Z. marina from the northern Pacific (Invers et al., 2001) at 0.56 mmol L$^{-1}$ and the North Sea at a background CO$_2$ concentration of about 8 μmol L$^{-1}$ (Sand-Jensen and Gordon, 1984) at 0.60 mmol L$^{-1}$ are substantially lower than the value of 1.18 mmol L$^{-1}$ reported here (Table 2). In contrast, the $K_{\text{HCO}_3}$ DIC concentration for Z. marina from the North Sea off the Swedish coast was 1.60 mmol L$^{-1}$ (Hellblom et al., 2001) is
predicted to, under the experimental conditions of pH 8.1, 20°C and a salinity of 30, is equivalent to a HCO\(_3\) concentration of 1.44 mmol L\(^{-1}\), slightly greater than our estimate. Recent estimates of K\(_{\text{HCO}_3}\) concentrations of DIC by Capo-Bauça et al. (2022) at 0.73 mmol L\(^{-1}\) for *P. oceanica* and 1.47 mmol L\(^{-1}\) for *Z. marina* (roughly 0.63 and 1.26 mmol L\(^{-1}\) HCO\(_3\); if the pH was 8.0) are very similar to ours at 0.62 and 1.18 mmol L\(^{-1}\) for *P. oceanica* and *Z. marina* respectively. Other seagrass species have also been estimated to have high K\(_{\text{HCO}_3}\) values for HCO\(_3\) such as *Thalassia testudinum* (>1.22 mmol L\(^{-1}\); Durako, 1993)). Despite these differences, the reported K\(_{\text{HCO}_3}\) concentrations for HCO\(_3\) are below ambient concentrations in seawater and even with the higher K\(_{\text{HCO}_3}\) values, such as those reported here, ambient concentrations of HCO\(_3\) are between 65 and 86% saturated for *Z. marina* and *P. oceanica* respectively.

Three possible, not mutually exclusive, mechanisms have been suggested to be involved in HCO\(_3\) use in seagrasses (Beer et al., 2002; Beer et al., 2006; Larkum et al., 2017). The first mechanism that is widespread, involves the maintenance of chemical equilibrium concentrations of CO\(_2\) close to the plasmalemma by a periplasmic carbonic anhydrase that can be inhibited by acetazolamide (Koch et al., 2013; Borum et al., 2016; Capo-Bauça et al., 2022). Photosynthesis by *P. oceanica* and *Z. marina* are sensitive to the inhibitor acetazolamide (Invers et al., 1999; Hellblom et al., 2001) but *P. oceanica* was inhibited to a greater extent than *Z. marina* (Capo-Bauça et al., 2022). The presence of two periplasmic CAs (KMZ64507 and KMZ75401) was confirmed in *Z. marina* by our sequence analysis. In this species, a permeable inhibitor, ethoxyzolamide, had an additional extrusion at the plasmalemma that converts HCO\(_3\) to CO\(_2\), possibly accelerated by external carbonic anhydrase, and can be inhibited by buffers (Uku et al., 2005; Beer et al., 2006; Borum et al., 2016). *Z. marina* was more strongly inhibited by TRIS buffer than *P. oceanica* (Capo-Bauça et al., 2022). The third mechanism involves proton extrusion at the plasmalemma that converts HCO\(_3\) to CO\(_2\), possibly accelerated by external carbonic anhydrase, and can be inhibited by buffers (Fernandez et al., 1999; Rubio and Fernández, 2019).

In marine macroalgae (Beer, 1994) and the freshwater angiosperm *Ottelia alismoides* (Huang et al., 2020), there is evidence that an anion exchange protein, SLC4, is involved in HCO\(_3\) use since photosynthesis can be inhibited by DIDS (4,4'-diisothiocyanatostilbene-2,2'-disulfonate). In contrast, at pH 8.1 DIDS had no effect on rates of photosynthesis in *Z. marina* (Hellblom et al., 2001; Capó-Bauça et al., 2022). Our analysis of the *Z. marina* genome shows that it possesses two genes for a SLC4 protein. The discrepancy between the lack of DIDS sensitivity and the presence of SLC4 in the genome could be explained by the predicted location of the two SLC4 proteins in the chloroplast. SLC4 proteins have also been found in the chloroplast membranes of the marine diatom *Phaeodactylum tricornutum* (Matsuda et al., 2017) and predicted in the chloroplast of the freshwater diatom *Asterionella formosa* (Maberly et al., 2021). *Z. marina* differs from *O. alismoides* (Huang et al., 2020) where SLC4 is periplasmic. *P. oceanica* is slightly inhibited by DIDS (Capo-Bauça et al., 2022) and it will be interesting to determine if SLC4 is present in its genome and where this protein is located.

Freshwater plants that are able to use HCO\(_3\) have a higher K\(_{\text{HCO}_3}\) for CO\(_2\) and a lower slope of photosynthesis rate vs CO\(_2\) concentration than freshwater plants restricted to CO\(_2\) (Maberly and Madsen, 1998). This is one of the ‘costs’ of using HCO\(_3\), in addition to an energy cost (Raven et al., 2014) that gives a disadvantage to using HCO\(_3\) in some ecological situations. The affinities of seagrasses and freshwater plants for CO\(_2\) based on the slope of photosynthesis vs concentration of CO\(_2\) (Table 2) converted to a dry weight basis, using values in Supplementary Table 4, were compared. The slope (as \(\mu\text{mol g}^{-1} \text{DW s}^{-1} \text{L mmol}^{-1} \text{CO}_2\)) for *P. oceanica* at 2.51 is similar to the mean for freshwater plants restricted to CO\(_2\) at 2.41 (Maberly and Madsen, 1998), while the slope for *Z. marina* at 0.33 is four-times lower than the mean slope for freshwater plants able to use HCO\(_3\) at 1.35. For freshwater plants, the lower affinity for CO\(_2\) in species able to use HCO\(_3\), compared to those restricted to CO\(_2\), was caused by a higher internal resistance to CO\(_2\) exchange (Madsen and Maberly, 2003). This was suggested to reduce losses of CO\(_2\) from the leaf produced by active uptake of HCO\(_3\).
is predicted to increase 1.46-fold from 9 to 36 μM CO₂ as a result of a 9.29-fold and 1.02-fold increase in the CO₂ and HCO₃⁻-dependent rate respectively. The overall increase is similar to the increase of three species of tropical seagrasses of 1.49-fold to 1.68-fold over a similar CO₂ concentration range based on the regressions of photosynthesis rate to CO₂ partial pressure in Ow et al. (2015). In contrast, the photosynthesis rate of P. oceanica would increase 3.22-fold from 9 to 36 μM CO₂ as a result of a 6.32-fold and 1.05-fold increase in the CO₂ and HCO₃⁻-dependent rate respectively. This suggests that this species may benefit particularly from rising concentrations of CO₂ (Figure 3). This is the result of a higher affinity and capacity to use CO₂, but a lower capacity to use HCO₃⁻ in P. oceanica than in Z. marina (Table 2). Unlike Z. marina, future projections suggest that CO₂ will become the major source of inorganic carbon in P. oceanica (Figure 3).

Although increased atmospheric CO₂ concentration will increase the concentration of CO₂ in water, increased water temperature will reduce CO₂ solubility by about 5% for a temperature increase of 2°C. Greater effects reducing the benefit of increased inorganic carbon availability are likely to result from interaction with other environmental factors. For example, limitation of photosynthesis by light or temperature (Maberly, 1985) or nutrients will decrease inorganic carbon limitation. In addition, Rubio et al. (2020) showed that there was an interaction between nitrate uptake and bicarbonate uptake in P. oceanica. High concentrations of HCO₃⁻ led to loss of cytosolic nitrate via S-type anion channels, which could lead to, or increase, nitrogen limitation.

A mystery surrounds the reason for the very small numbers of plant species in the ocean. Suggestions include difficulties of pollination, especially given the lack of co-evolution with insects (Van der Hage, 1996), competition with macroalgae (Maberly and Gontero, 2018), toxicity of sulfide in the sediment (van der Heide et al., 1996) but not salinity per se (Touchette, 2007). Seagrasses are believed to have invaded the ocean about 100 to 140 Mya, probably from freshwater descendants given their phylogeny (Les et al., 1997; Les and Tippery, 2013). At this time, the atmospheric CO₂ partial pressure is believed to have been 860 to 1000 ppm (Foster et al., 2017) while ocean carbonate alkalinity was similar to that today (Zeebe and Tyrrell, 2019). Assuming an unchanged physiology, CO₂ concentrations in the Cretaceous will have been slightly more favourable for seagrasses than those today. Ironically, these concentrations are similar to some projections for the end of the century. While CO₂ concentrations in the oceans are close to atmospheric equilibrium, in freshwaters, especially rivers, CO₂ concentrations can be extremely depleted, but more frequently exceed atmospheric CO₂ equilibrium (Cole et al., 1994; Maberly et al., 2013). Air-equilibrium concentrations of CO₂ in the ocean may therefore have reduced the fitness of plants invading from fresh waters.

**Perspectives**

A number of physiological uncertainties need to be addressed. First, further work is needed to determine if the different reported Kᵢ₃ concentrations for HCO₃⁻ (in the absence of buffers) are the result of genotypic or phenotypic differences in the plant material, the measurement method or how the data were analysed. Secondly, further research is needed to determine the extent to which seagrasses can acclimate to variable CO₂. Rapid acclimation would be one explanation for the lower HC₃⁻ compensation during the pH-drift of up to 60 hours compared to the concentrations determined from the short-term kinetic measurements. Thirdly, although there is strong circumstantial evidence for a CCM in many seagrass species, evidence is currently lacking that shows that the concentration of CO₂ around Rubisco is elevated above the external concentration (Larkin et al., 2017). Fourthly, further work is needed to establish if an anion exchange protein such as SLC4 is involved in the photosynthesis of Z. marina and other species of seagrass and where it is located. Fifthly, it is unclear why the affinity to CO₂ of seagrasses that are able to use HCO₃⁻ differs among themselves and from freshwater plants. Finally, more research is needed to compare structural difference between the leaves of seagrasses and freshwater plants and the mechanisms and location where HCO₃⁻ is converted to CO₂ and how this interacts with the different carbonate chemistry in the oceans compared to fresh waters.

The differential ability of seagrasses to exploit inorganic carbon reserves may control their future ecological success. The growing availability of genomic data for seagrasses (Olsen et al., 2016) is becoming a powerful tool to understand differential species responses to environmental change, especially when combined with more traditional approaches (Davey et al., 2016). This will provide more insights into the mechanisms supporting inorganic carbon uptake of individual species and increase our understanding of the different carbon uptake capabilities among species and their ecological consequences under environmental change.

**Data availability statement**

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

**Author contributions**

SM and BG devised the work plan and carried out the experiments. AS made the stable carbon isotope measurements. SM and BG analyzed the data and wrote the manuscript. All
authors contributed to the article and approved the submitted version.

Funding

BG is supported by the Centre National de la Recherche Scientifique, Aix-Marseille Université, and currently by the Agence Nationale de la Recherche (OCEANIA, ANR-21-CE20-0029-01). SM’s work was supported by a visiting scholarship from the University of Aix-Marseille and a ‘Make our Planet Great Again’ scholarship from the French Government.

Acknowledgments

We thank Sandrine Ruitton for help and advice and for collecting *P. oceanica* with the crew of the Antedon II and the CNRS divers. Veronique Receveur-Brechet is thanked for help with field work.

References

Beers, S. (1994). Mechanisms of inorganic carbon acquisition in marine macroalgae (with special reference to the chlorophyta). *Prog. Physiol. Res.* 10, 179–207.

Beers, S., Axelson, L., and Bjork, M. (2006). Modes of photosynthetic bicarbonate utilisation in seagrasses, and their possible roles in adaptation to specific habitats. *Bol. Mar. Mediterr.* 13, 3–7.

Beers, S., Bjork, M., Hellblom, F., and Axelson, L. (2002). Inorganic carbon utilisation in marine angiosperms (seagrasses). *Funct. Plant Biol.* 29, 349–354. doi: 10.1071/FP01185

Berg, P., Delgard, M. L., Polsenære, P., Meglatherby, K. J., Doney, S. C., and Berger, A. C. (1999). Dynamics of benthic metabolism, O₂– and pCO₂ in a temperate seagrass meadow. *Limnol. Oceanogr.* 64, 2586–2604. doi: 10.1002/ino.11236

Bjork, M., Weil, A., Semesi, S., and Beer, S. (1997). Photosynthetic utilisation of inorganic carbon by seagrasses from Zanzibar, East Africa. *Mar. Biol.* 129, 363–366. doi: 10.1007/s002270050176

Borum, J., Pedersen, O., Kotula, L., Fraser, M. W., Statton, J., Colmer, T. D., et al. (2016). Photosynthetic response to globally increasing CO₂ of co-occurring temperate seagrasses. *Plant Cell Environ.* 39, 1240–1250. doi: 10.1111/pce.12658

Burnell, O. W., Connell, S. D., Irving, A. D., Watling, J. R., and Russell, B. D. (2014). Contemporary reliance on bicarbonate acquisition predicts increased growth of seagrass *Amphibolus antarctica* in a high-CO₂ world. *Conserv. Physiol.* 2. doi: 10.1093/comphys/cou052

Capó-Baqué, S., Intergoz, C., Aguilo-Nicolau, P., and Galmés, J. (2022). Correlative adaptation between rubisco and CO₂-concentrating mechanisms in seagrasses. *Nat. Plants* 8, 706–716. doi: 10.1038/s41477-022-01171-5

Clement, R., Dimnet, L., Maberly, S. C., and Gontero, B. (2016). The nature of the CO₂-concentrating mechanisms in a marine diatom, *Thalassiosira pseudonana*. *New Phytol.* 208, 1417–1427. doi: 10.1111/nph.13978

Cole, J. J., Caraco, N. F., Kling, G. W., and Kratz, T. K. (1994). Carbon-dioxide supersaturation in the surface waters of lakes. *Science* 265, 1568–1570. doi: 10.1126/science.265.5178.1568

Davye, P. A., Pernice, M., Sahlok, G., Larkum, A., Lee, H. T., Golizc, A., et al. (2016). The emergence of molecular profiling and omics techniques in seagrass biology; furthering our understanding of seagrasses. *Funct. Integr. Genomics* 16, 465–486. doi: 10.1007/s10241-016-0501-4

Doney, S. C., Fabry, V. J., Feely, R. A., and Kleypas, J. A. (2009). Ocean acidification: The other CO₂ problem. *Ann. Rev. Mar. Sci.* 1, 169–192. doi: 10.1146/annurev.marine.010908.163834

Drechsel, Z., Sharkia, R., Cabanthick, Z. I., and Beer, S. (1993). Bicarbonate uptake in the marine macroalga *Ulva* sp is inhibited by classical probes of anion-exchange by red-blood-cells. *Plants* 191, 34–40. doi: 10.1007/BF00248893

Duarte, C. M. (2017). Reviews and syntheses: Hidden forests, the role of vegetated coastal habitats in the ocean carbon budget. *Biogeoosciences* 14, 301–310. doi: 10.5194/bg-14-301-2017

Duarte, C. M., Middborg, J. I., and Caraco, N. (2005). Major role of marine vegetation on the oceanic carbon cycle. *Biogeoosciences* 2, 1–8. doi: 10.5194/bg-2-1-2005

Durako, M. J. (1993). Photosynthetic utilization of CO₂(aq) and HCO₃⁻ in *Thalassia-testudinum* (Hydrocharitaceae). *Mar. Biol.* 115, 373–380. doi: 10.1007/BF00349834

Fernandez, J. A., Garcia-Sanchez, M. J., and Felle, H. H. (1999). Physiological evidence for a proton pump and sodium exclusion mechanisms at the plasma membrane of the marine angiosperm *Zostera marina* I. *J. Exp. Bot.* 50, 1763–1768. doi: 10.1093/jxb/50.341.1763

Fernandez, P. A., Hurd, C. L., and Rolleda, M. Y. (2014). Bicarbonate uptake via an anion exchange protein is the main mechanism of inorganic carbon acquisition by the giant kelp *Macrocystis pyrifera* (laminariaceae, phaeophyceae) under variable pH. *J. Phycol.* 50, 998–1008. doi: 10.1111/jpy.12247

Fernandez, P. A., Rolleda, M. Y., Rautenberger, R., Hurd, C. L., et al (2018). Carbonic anhydrase activity in seaweeds: overview and recommendations for measuring activity with an electrometric method, using *Macrocystis pyrifera* as a model species. *Mar. Biol.* 165, 88. doi: 10.1007/s00227-018-3348-5

Foster, G. L., Roer, D. L., and Lunt, D. J. (2017). Future climate forcing potentially without precedent in the last 420 million years. *Nat. Commun.* 8, 14845. doi: 10.1038/ncomms14845

Galmés, J., Hermida-Carrera, C., Laanisto, L., and Ninemets, U. (2016). A compendium of temperature responses of rubisco kinetic traits: variability among and within photosynthetic groups and impacts on photosynthesis modeling. *J. Exp. Bot.* 67, 5067–5091. doi: 10.1093/jxb/erw267

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher’s note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2022.936716/full#supplementary-material
Galmean, I., Turpin, C. F., Paule, M. A., von Ende, C., Grun, S., et al. (2014). Reproductive success of the common seagrass Zostera marina. Aquat. Bot. 132, 37–42. doi: 10.1016/j.aquabot.2014.03.004

Hunes, B., and Maat, M. G. (2014). Fast response of calcification in the shallow-water coral Porites astreoides. J. Exp. Mar. Biol. Ecol. 455, 1–11. doi: 10.1016/j.jembe.2014.08.001

Koch, M., Moriyama, K., Inamori, M., Chen, H. Y., et al. (2014). Comparative analysis of the transcriptome and proteome of Porites astreoides and Acropora millepora in response to temperature and ultraviolet-B radiation. Mar. Biol. 161, 767–783. doi: 10.1007/s00227-014-2428-5

Larkum, A. W. D., Brown, K. E., and Parry, M. J. (2014). “Photosynthesis and metabolism in seagrasses at the cellular level,” in Seagrasses: Biology, ecology and conservation. Eds. A. W. D. Larkum, J. J. Orth and C. M. Duarte (Berlin: Springer Publishing), 307–347.

Larkum, A. W. D., Zerba, O. J., and Parry, M. J. (2014). “Photosynthesis and metabolism in seagrasses at the cellular level,” in Seagrasses: Biology, ecology and conservation. Eds. A. W. D. Larkum, J. J. Orth and C. M. Duarte (Berlin: Springer Publishing), 307–347.

Larkum, A. W. D., Zerba, O. J., and Parry, M. J. (2014). “Photosynthesis and metabolism in seagrasses at the cellular level,” in Seagrasses: Biology, ecology and conservation. Eds. A. W. D. Larkum, J. J. Orth and C. M. Duarte (Berlin: Springer Publishing), 307–347.

Larkum, A. W. D., Zerba, O. J., and Parry, M. J. (2014). “Photosynthesis and metabolism in seagrasses at the cellular level,” in Seagrasses: Biology, ecology and conservation. Eds. A. W. D. Larkum, J. J. Orth and C. M. Duarte (Berlin: Springer Publishing), 307–347.
