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Potential impact of DOM accumulation on $f\mathrm{CO}_2$ and carbonate ion computations in ocean acidification experiments

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Abstract. The internal consistency of measurements and computations of components of the CO_2-system, namely total alkalinity (A_T), total dissolved carbon dioxide (C_T), CO_2 fugacity ($f\mathrm{CO}_2$) and pH, has been confirmed repeatedly in open ocean studies when the CO_2 system had been over determined. Differences between measured and computed properties, such as $\Delta f\mathrm{CO}_2$ ($= f\mathrm{CO}_2$ (measured) $- f\mathrm{CO}_2$ (computed from A_T and C_T)) $/ f\mathrm{CO}_2$ (measured) $\times 100$), are usually below 5 %. Recently, Hoppe et al. (2012) provided evidence of significantly larger $\Delta f\mathrm{CO}_2$ in some experimental setups. These observations are currently not well understood. Here we discuss a case from a series of phytoplankton culture experiments with $\Delta f\mathrm{CO}_2$ of up to about 25 %. $\Delta f\mathrm{CO}_2$ varied systematically during the course of these experiments and showed a clear correlation with the accumulation of dissolved organic matter (DOM).

Culture and mesocosm experiments are often carried out under high initial nutrient concentrations, yielding high biomass concentrations that in turn often lead to a substantial build-up of DOM. In such experiments, DOM can reach concentrations much higher than typically observed in the open ocean. To the extent that DOM includes organic acids and bases, it will contribute to the alkalinity of the seawater contained in the experimental device. Our analysis suggests that whenever substantial amounts of DOM are produced during the experiment, standard computer programmes used to compute CO_2 fugacity can underestimate true $f\mathrm{CO}_2$ significantly when the computation is based on A_T and C_T. Unless the effect of DOM-alkalinity can be accounted for, this might lead to significant errors in the interpretation of the system under consideration with respect to the experimentally applied CO_2 perturbation. Errors in the inferred $f\mathrm{CO}_2$ can misguide the development of parameterisations used in simulations with global carbon cycle models in future CO_2-scenarios. Over determination of the CO_2-system in experimental ocean acidification studies is proposed to safeguard against possibly large errors in estimated $f\mathrm{CO}_2$.

1 Introduction

One of the largest anthropogenic perturbations of the marine environment is caused by the invasion of anthropogenic CO_2 into the ocean (Sabine et al., 2004). CO_2 entering the ocean changes the seawater carbonate equilibrium, decreases pH and the concentration of carbonate ions ($[\mathrm{CO}_3^{2-}]$) and increases the ocean $f\mathrm{CO}_2$ (Bates, 2001; Keeling et al., 2004; Byrne et al., 2010). Changes in seawater chemistry are expected to impact individual species as well as communities and ecosystems (Raven et al., 2005). The potential for marine organisms, communities and ecosystems to adapt to these changes is not well understood, making the ocean acidification issue a high priority field of research (Doney et al., 2009). An obvious approach is experimental manipulation in which individual species or even whole ecosystems are studied under high $f\mathrm{CO}_2$ conditions expected for the future (Orr et al., 2005), as well as low $f\mathrm{CO}_2$ conditions known to predominate during the preindustrial era (Barnola et al., 1987).

Characterising the seawater carbon dioxide system and its individual components, in the ocean as well as in experimental setups, requires high quality measurements of at least two out of the four measurable properties of the carbon dioxide system, namely total alkalinity (A_T), total dissolved carbon dioxide (C_T), CO_2 fugacity ($f\mathrm{CO}_2$) and pH. Instrumentation and protocols have significantly developed during the

recent two decades allowing for very precise measurements of the marine CO_2 system (DOE, 1994; Dickson et al., 2007; Dickson, 2010). The internal consistency of these measurements and computations of components of the CO_2-system has been studied repeatedly when the CO_2 system had been over determined, i.e., when more than two types of measurements were available (McElligott et al., 1998; Wanninkhof et al., 1999; Lee et al., 2000; Millero et al., 2002, 2006). For the recommended choice of thermodynamic constants, the degree of inconsistency (computation error), expressed as $\Delta X = (X_{\text{measured}} - X_{\text{calculated}}) / X_{\text{measured}} \times 100$, of high quality oceanic measurements is usually well below 5 % for $f\text{CO}_2$ (Lee et al., 2000).

Recently, Hoppe et al. (2012) provided evidence for much higher and systematic differences of measured and computed $p\text{CO}_2$ from experimental manipulation of North Sea water prepared for studies of ocean acidification on plankton. These authors measured $p\text{CO}_2$ as well as computed it from the following pairs of measured variables, $A_T + C_T$, $A_T + \text{pH}$, and $C_T + \text{pH}$. The largest deviations from measured $p\text{CO}_2$ is obtained when computing $p\text{CO}_2$ from A_T and C_T, with respective $\Delta p\text{CO}_2$ values of 25 % (± 0.3; geometric mean $\pm$ variance; $N = 12$, computed from data from Table 1 of Hoppe et al., 2012, Appendix). Smaller deviations where found when calculating $p\text{CO}_2$ from C_T and pH ($\Delta X = 6.0 \pm 0.3$ %; $N = 11$) or A_T and pH ($\Delta X = 4.6 \pm 0.4$ %; $N = 11$). $\Delta p\text{CO}_2$ was independent of the method of seawater manipulation (bubbling, acid/base addition) and the magnitude of $p\text{CO}_2$. Hoppe et al. (2012) also compiled data from the limited number of ocean acidification experimental studies where the CO_2-system was overdetermined, providing evidence that a bias in $p\text{CO}_2$ values computed from A_T and C_T of about 30 % relative to reference measurements may be more widespread. While the cause of this bias is currently not understood, it is critical to be aware of possibly large errors in $f\text{CO}_2$ computed from A_T and C_T in order to avoid misinterpretations of experimental results and also in order to better constrain estimated values of $f\text{CO}_2$ in future experiments addressing ocean acidification.

In a commentary to the Hoppe et al. (2010), Tyrrell (2010) proposed that one possible explanation of the high $\Delta p\text{CO}_2$ values might be related to an unrecognised contribution of dissolved organic matter (DOM), or more explicitly organic acids/bases, to measured A_T and the inability of implementations of the standard seawater chemical model (i.e., available software tools) used for carbon system calculations to correctly account for this effect when computing $p\text{CO}_2$. Such software implies established models of seawater chemistry, in particular, concerning the involved chemical species and related thermodynamic constants. Weak organic acids and bases are formally part of the definition of alkalinity (Dickson, 1981; see also Sect. 3.2 and equation 1). Their quantitative contribution to alkalinity is, however, usually assumed to be of minor importance. Hence, these substances are indicated often only implicitly in equations defining seawater

alkalinity (e.g., ellipsis in Eq. 1). Usually these substances also are neglected in the algorithms applied in the respective software tools.

$$A_T = \left[\text{HCO}_3^-\right] + 2\left[\text{CO}_3^{2-}\right] + \left[B\left(\text{OH}\right)^-\right] + \left[\text{OH}^-\right] \quad (1)$$
$$+ \left[\text{HPO}_4^{2-}\right] + 2\left[\text{PO}_4^{3-}\right] + \left[\text{SiO}\left(\text{OH}\right)_3^-\right] + \left[\text{NH}_3\right]$$
$$+ \left[\text{HS}^-\right] + ... - \left[\text{H}^+\right] - \left[\text{HSO}_4^-\right] - \left[\text{HF}\right] - \left[\text{H}_3\text{PO}_4\right] - ...$$

Based on recently published experimental work (Kim and Lee, 2009), we explore here the role of DOM accumulation during a set of culture experiments on the computation of $f\text{CO}_2$ and the carbonate ion concentration, and how the neglect of DOM impacts on sea water alkalinity can affect the interpretation of ocean acidification experiments. We acknowledge that this does not add new knowledge about the CO_2 system, which is well understood theoretically (e.g., Dickson, 1981). However, we wish to raise awareness of potentially large systematic errors when porting short-cut CO_2 calculations, which work well under open-ocean conditions, to experimental settings often used in ocean acidification research.

2 Experiments and analytical methods

In order to demonstrate the effect of freshly produced DOM on carbonate system computations, we make use of data from a series of phytoplankton culture experiments published recently and described in detail by Kim and Lee (2009). In short, strains of three phytoplankton species (*Prorocentrum minimum*, *Skeletonema costatum* and *Chaetoceros curvisetus*) were collected from Jangmok Bay, Korea (34.6° N, 128.5° E) and incubated at 20 °C in filtered and autoclaved seawater augmented with nutrients (initial concentrations of $70\,\mu\text{mol}\,l^{-1}$ nitrate, $5\,\mu\text{mol}\,l^{-1}$ phosphate and $50\,\mu\text{mol}\,l^{-1}$ silicate). In order to extend the growth period and duration of the experiment, a low photon flux density ($10\,\mu\text{mol photons}\,\text{m}^{-2}\,\text{s}^{-1}$; 12 : 12 light/dark cycle) yielding low specific growth rates was chosen. Phytoplankton cultures were periodically bubbled with air containing 5 % CO_2 in order to maintain the solution pH in the range 7.8–8.2, within which spectrophotometric pH measurement applied in this study is optimal (Clayton and Byrne, 1993). Prior to each sampling the culture solution was mechanically mixed, minimising the possibility of sampling bias due to solution inhomogeneity.

In order to quantify the accumulation of DOM over the course of the experiment dissolved organic carbon (DOC) was measured (Kim and Lee, 2009). For DOC analysis, seawater samples were filtered through a pre-combusted filter (nominal pore size $0.7\,\mu\text{m}$; Whatman GF/F), filtrates were collected in precombusted glass vials (Corning 430052), acidified with 10 % H_3PO_4 solution and purged (10 min)

with ultrapure N_2 gas in order to remove inorganic carbon. Sub-samples (triplicates) were injected into the combustion tube of a Shimadzu TOC-VCPH total organic carbon analyser (Shimadzu Scientific Instruments, Kyoto, Japan) containing a platinum catalyst at 650 °C, for oxidation of DOC to CO_2. CO_2-detection was with a non-dispersive infrared detector. The total alkalinity (A_T) and total inorganic carbon (C_T) values were determined using potentiometric acid titration and coulometric titration in a VINDTA system (Marianda, Kiel, Germany). The measurement precisions were $\pm 1.5\,\mu\text{mol kg}^{-1}$ for A_T and $\pm 2\,\mu\text{mol kg}^{-1}$ for C_T (for details see Kim and Lee, 2009). Kim and Lee (2009) evaluated A_T based on the nonlinear curve-fitting of titration data (Dickson, 1981; DOE, 1994; K. Lee, personal communication, 2012).

Measurements of seawater pH were made to a precision of ± 0.001 at 25 °C using a double-wavelength spectrophotometric procedure and m-cresol purple indicator, following Clayton and Byrne (1993). Nitrate was measured following a manual procedure (Hansen and Koroleff, 1999). All A_T and nutrient measurements were performed on seawater samples filtered (nominal pore size 0.7 μm; Whatman GF/F) under a slight vacuum pressure (< 17 kPa) to remove all phytoplankton cells. Filtration of A_T was carried out in order to exclude any potential contribution of particle surfaces to measured alkalinity (Kim et al., 2006). Measurements of C_T and pH were performed on unfiltered seawater samples to avoid CO_2 gain or loss during the filtration process (see Supplement in Kim and Lee, 2009).

No direct measurements of $f\text{CO}_2$ are available from this experiment. $f\text{CO}_2$ (and $[CO_3^{2-}]$, the carbonate ion concentration) are computed independently from the following pairs of measurements: $C_T + \text{pH}$, $A_T + \text{pH}$, and $A_T + C_T$. For all computations we apply the Matlab-version of CO2SYS (Lewis and Wallace, 1998; van Heuven et al., 2009; http://cdiac.ornl.gov/oceans/co2rprt.html), using carbonic acid dissociation constants of Mehrbach et al. (1973), as refitted by Dickson and Millero (1987), and other ancillary thermodynamic constants (Millero, 1995). In the absence of measured $f\text{CO}_2$ values, we compute e.g., $\Delta f\text{CO}_{2(A_T,C_T)} = (f\text{CO}_{2\,(\text{reference})} - f\text{CO}_{2\,(A_T,C_T)})/f\text{CO}_{2\,(\text{reference})} \times 100$, using $f\text{CO}_{2\,(C_T,\text{pH})}$ as the reference, $f\text{CO}_{2\,(\text{reference})}$. This choice of the $f\text{CO}_{2\,(\text{reference})}$ is a priori derived from our working hypothesis that the significant $\Delta f\text{CO}_2$, which we report is due to the alkalinity effect from DOM (organic acids/bases) accumulating during the experiments. Organic acids/bases are not known to affect the analytical methods applied to measure pH and C_T in the study of Kim and Lee (2009). A posteori the choice of the reference is justified by the thought experiment and related model simulations presented in Sect. 3.2. We compute $\Delta[CO_3^{2-}]_{(A_T,C_T)} = ([CO_3^{2-}]_{(\text{reference})} - [CO_3^{2-}]_{(A_T,C_T)})/[CO_3^{2-}]_{(\text{reference})} \times 100$, accordingly.

3 Results

3.1 Jangmok Bay phytoplankton cultures

During the incubation, the NO_3^- concentration in all three cultures (*P. minimum*, *S. costatum* and *C. curvisetus*) dropped from about $70\,\mu\text{mol l}^{-1}$, i.e., nutrient concentrations much higher than found in the open ocean, to values below the detection limit ($0.2\,\mu\text{mol l}^{-1}$) approximately 2 weeks after the incubation commenced (Fig. 1a; Kim and Lee, 2009). DOC increased during this period and continued to increase after NO_3^- concentrations became (and remained) depleted (Fig. 1b), as did measured A_T (Kim and Lee, 2009). ΔDOC, the amount of accumulated DOC, was as high as $50\,\mu\text{mol l}^{-1}$ at the end of the experiments. Due to the intentional periodical bubbling with CO_2-enriched air (see methods section), C_T concentrations increased over the course of the experiment (not shown), rather than decreased as observed regularly in micro- or mesocosm experiments where the pH is allowed to drift freely (e.g., Goldman, 1999; Riebesell et al., 2007). During the experiment $\Delta f\text{CO}_2$ increased with time while $\Delta[CO_3^{2-}]$ decreased (Fig. 2), reaching values as high (or low) as 23 % and −24 % for $\Delta f\text{CO}_2$ and $\Delta[CO_3^{2-}]$, respectively. Initial values of $\Delta f\text{CO}_2$ ($\Delta[CO_3^{2-}]$) were 2 to 6 % (−2 to −5 %), which is similar to ranges of ΔX values observed during open ocean consistency studies (Lee et al., 2000). There is a clear relationship of $\Delta f\text{CO}_2$ and $\Delta[CO_3^{2-}]$ with the concentration of DOC accumulating during the experiment (ΔDOC, Fig. 3), not surprising in view of the tight relationship between ΔDOC and the difference between measured A_T and A_T computed from pH and C_T, ($A_{T(\text{measured})} - A_{T(C_T,\text{pH})}$). As discussed in detail by Kim and Lee (2009), ($A_{T(\text{measured})} - A_{T(C_T,\text{pH})}$) increased in tight correlation with nitrate consumption, with final values of about $20\,\mu\text{mol kg}^{-1}$ (*S. costatum* and *C. curvisetus* cultures) and $42\,\mu\text{mol kg}^{-1}$ (*P. minimum* culture). Slopes of ($A_{T(\text{measured})} - A_{T(C_T,\text{pH})}$) and ΔDOC ranged between 0.82 and 1.21.

3.2 Theoretical case studies

In this section, we quantitatively explore the potential role of organic acids/bases for CO_2-system computations by means of a thought experiment and numerical simulations. We consider a water sample with a total alkalinity of $2400\,\mu\text{mol kg}^{-1}$, a prescribed potential DOM-alkalinity from weak organic acids/bases of $50\,\mu\text{mol kg}^{-1}$, $S = 35$, $T = 25\,°\text{C}$, total-PO_4 and total-$\text{Si(OH)}_4 = 0\,\mu\text{mol kg}^{-1}$ of which subsamples have been equilibrated with $f\text{CO}_2$ between 100 and 3000 μatm. The high-end range of $f\text{CO}_2$ is relevant in low-oxygen waters, which had been equilibrated with the atmosphere under current conditions (Brewer and Peltzer, 2009).

Furthermore, we have coded a modified version of the CO2SYS-programme, which accounts for the contribution of organic acids/bases to total alkalinity when computing
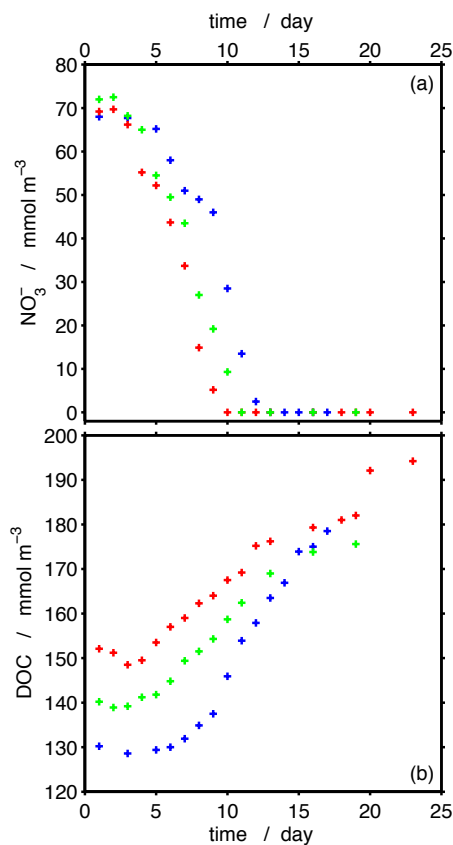
Fig. 1. Time course development of nitrate (mmol m^{-3}; **a**) and DOC (mmol m^{-3}; **b**) concentrations during three phytoplankton culture experiments. Blue: *Prorocentrum minimum* (Dinoflagellate), red: *Skeletonema costatum* (Diatom), green: *Chaetoceros curvisetus* (Diatom).

Fig. 2. Time course of $\Delta f CO_2$ **(a)** and $\Delta[CO_3^{2-}]$ **(b)** during phytoplankton culture experiments. Colour code as in Fig. 1. $\Delta f CO_2$ and $\Delta[CO_3^{2-}]$ are computed as follows: $\Delta f CO_2 = (f CO_{2(C_T,pH)} - f CO_{2(A_T,C_T)}) / f CO_{2(C_T,pH)} \times 100$; $\Delta[CO_3^{2-}] = ([CO_3^{2-}]_{(C_T,pH)} - [CO_3^{2-}]_{(A_T,C_T)}) / [CO_3^{2-}]_{(C_T,pH)} \times 100$.

missing variables of the CO_2-system from known pairs of input variables (in the following CO2SYS-DOM) The code of CO2SYS-DOM is available from the GEOMAR publication repository (http://oceanrep.geomar.de/14871/). In particular, we add an explicit term $+[Org^-]$ to the definition of alkalinity (Eq. 1) representing weak organic acid-base pairs dissociated under conditions of seawater pH. Technically, this term is implemented in all relevant modules of CO2SYS-DOM. We compute $[Org^-]$ according to Eq. (2)

$$[Org^-] = [\sum Org] \times (K_{Org}/(K_{Org} + [H^+])) \qquad (2)$$

where $[\sum Org]$ is the prescribed potential DOM-alkalinity and $K_{Org} = 10^{-pK_{Org}}$. Until now, little is known about typical values or the distribution of pK_{Org} in seawater, except for humic acids (Cai et al., 1998). Having no explicit knowledge of pK_{Org} for freshly produced organic matter under experimental conditions, e.g., for the phytoplankton cultures presented above, we here simplify and assume that the dissociation of organic acids/bases can be described by a single dissociation constant and choose a pK well below seawater pH such that
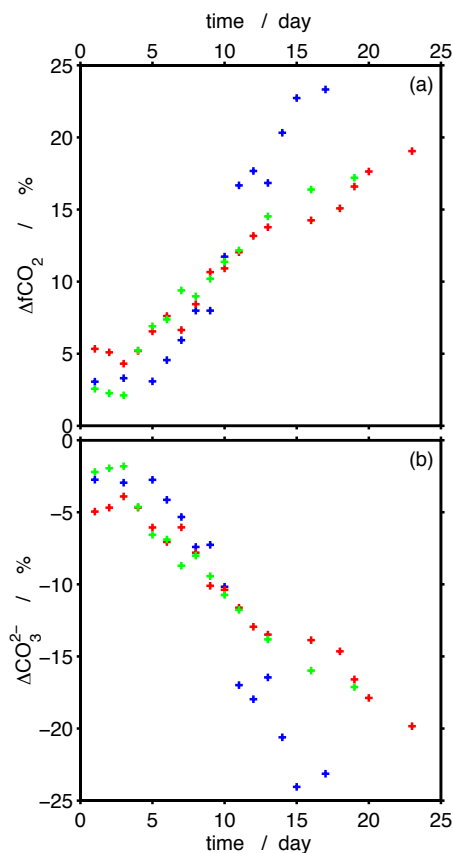
$[Org^-] \approx [\sum Org]$. Our approach is consistent with a similar ad hoc formulation used in Millero et al. (2002). Numerically, we implement a constant pK_{Org} of 4.6, independent of temperature and ionic strenght, as the standard case in CO2SYS-DOM. The sensitivity of our results to the choice of pK_{Org} will be discussed at the end of Sect. 3.2.

Using our virtual input data of A_T, prescribed $[\sum Org]$, S, T, total phosphate, total silicate and the given range of $f CO_2$, we compute C_T and pH for all $f CO_2$ subsamples using CO2SYS-DOM. In order to check the consistency of our A_T, $f CO_2$, C_T and pH data we compute $f CO_2$ from all possible pairs of input variables using CO2SYS-DOM and compare the diagnosed $f CO_2$ values against the $f CO_2$ values imposed in the thought experiment. We find that the imposed $f CO_2$, $f CO_{2(A_T,C_T)}$, $f CO_{2(C_T,pH)}$, and $f CO_{2(A_T,pH)}$ all agree with each other within 10^{-5} %. Note that in this section, $\Delta f CO_2$ is always computed using the imposed $f CO_2$ (which, in real experiments, would be the measured $f CO_2$) as the reference.

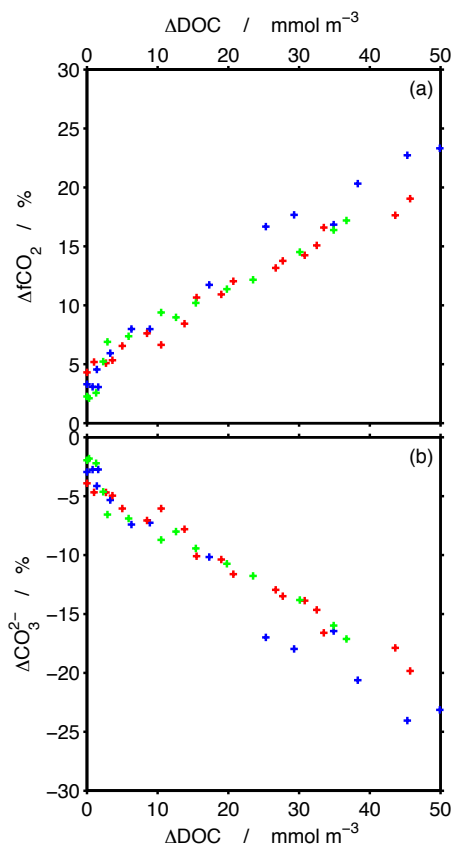**Fig. 3. (a)** Relationship between DOC accumulated during the experiment (ΔDOC) and **(a)** $\Delta f CO_2$ and **(b)** $\Delta[CO_3^{2-}]$. Colour code as in Fig. 1, $\Delta f CO_2$ and $\Delta[CO_3^{2-}]$ computed as explained in Fig. 2.

Fig. 4. Numerical simulations assuming a DOC-alkalinity of $50 \, \mu mol \, kg^{-1}$ (for details see Sect. 3.2). **(a)** $\Delta f CO_{2(A_T, C_T)}$ (solid line), $\Delta f CO_{2(A_T, pH)}$ (dashed line), and $\Delta f CO_{2(C_T, pH)}$ (dotted lines). **(b)** Relative anomalies (%) of $f CO_2$, $[H^+]$, $[HCO_3^-]$.

In the following, we will consider this virtual dataset like an over-determined CO_2-system dataset having all four possible measurements, i.e., C_T, A_T, $f CO_2$ and pH. We use these data as input to the computation of $f CO_2$ from all three possible input pairs. However, for doing so, we return to the standard procedure and do the following computations with the standard version of CO2SYS. Computing $\Delta f CO_2$ against $f CO_{2(imposed)}$ as the reference (Fig. 4a), we find that $f CO_{2(C_T, pH)}$ is in perfect agreement with $f CO_{2(imposed)}$. $\Delta f CO_{2(A_T, pH)}$ is small, negative and basically constant indicating that the $f CO_2$ computed from A_T and pH is a slight overestimate. $\Delta f CO_{2(A_T, C_T)}$ is positive, indicating that the computed $f CO_2$ is underestimated compared with $f CO_{2(imposed)}$, much larger and variable over the range of explored $f CO_2$. For the prescribed value of DOM-$A_T \Delta f CO_{2(A_T, pH)}$ is about -2.2 %, and $\Delta f CO_{2(A_T, C_T)}$ is about 15 % at low $f CO_2$. Moreover, $\Delta f CO_{2(A_T, C_T)}$ increases linearly up to 1000 µatm and shows an approximately constant value of about 28 % between 2000 and 3000 µatm, before it decreases again at an even higher $f CO_2$ (not shown). For the conditions assumed here, the maximum of 28.6 % is observed at about 2700 µatm.
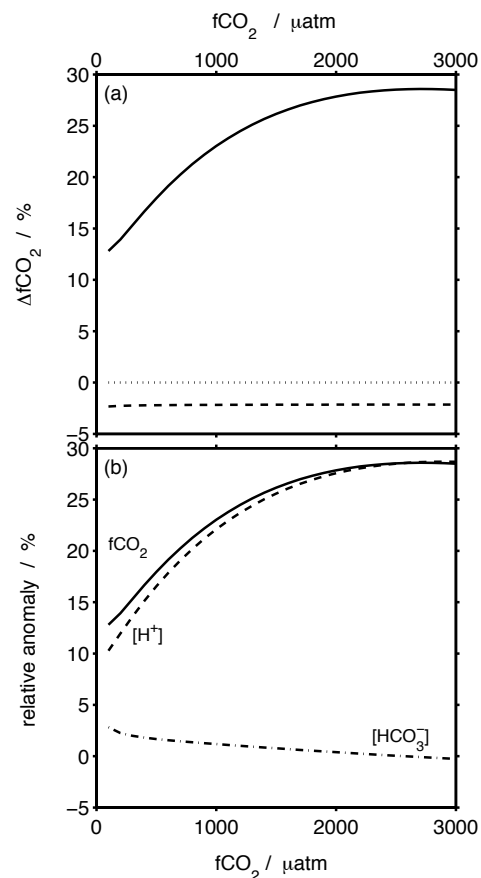
The observed behaviour is easily understood when considering the equations used to compute $f CO_2$. Independent of the input variables, $f CO_2$ is computed from an estimate of the CO_2 concentration, $[CO_2^*]$, and the solubility coefficient of CO_2 in seawater, K_0, according to Eqs. (3) and (4):

$$f CO_2 = \frac{[CO_2^*]}{K_0} \tag{3}$$

$$[CO_2^*] = C_T \times \frac{[H^+]^2}{[H^+]^2 + K_1[H^+] + K_1 K_2} \tag{4}$$

where $[H^+]$ is the hydrogen ion concentration, with $[H^+] = 10^{-pH}$, K_1 and K_2 are the first and second dissociation constants of carbonic acid.

When C_T and pH are used as input variables, the computation of $[CO_2^*]$ is not affected by any uncertainty related to the DOM-alkalinity and, hence, $f CO_{2(C_T, pH)}$ equals the imposed $f CO_2$ (Fig. 4a, dotted line).

Considering the pair of A_T and pH as input variables to Eq. (3) requires an estimate of C_T, which is computed according to Eq. (5):

$$C_T = A_C \times \frac{[H^+]^2 + K_1[H^+] + K_1 K_2}{K_1[H^+] + 2K_1 K_2} \quad (5)$$

where A_C is the carbonate alkalinity, $A_C = [HCO_3^-] + 2[CO_3^{2-}]$, which is computed from $A_C = A_T - A_{minor}$. A_{minor} is the sum of all minor components of seawater alkalinity (Eq. 6), in particular, borate alkalinity, P-, Si-, S-, and F-alkalinity, $[OH^-]$, $[H^+]$, and eventually the DOM-alkalinity, indicated as ellipsis in Eq. (6).

$$
\begin{aligned}
A_{minor} = {}& \left[B(OH)^-\right] + [OH^-] + \left[HPO_4^{2-}\right] + 2\left[PO_4^{3-}\right] \quad (6) \\
& + \left[SiO(OH)_3^-\right] + [NH_3] + [HS^-] + ... \\
& - [H^+] - [HSO_4^-] - [HF] - [H_3PO_4] - ...
\end{aligned}
$$

Since in this case pH and A_T are both measured variables, all uncertainty in the computation of $f CO_2$ resides in the estimate of A_{minor}. In our thought experiment, the absolute error of A_{minor} is equivalent to the value of the effective DOM-alkalinity if this term cannot be quantified in the computation due to a lack (or ignorance) of respective data and pK values. The relative error (dashed line in Fig. 4a), i.e., $\Delta f CO_{2(A_T, pH)}$, scales with ΔA_C which in our idealised example ranges between -2.15% and -2.35% for high and low-end prescribed $f CO_2$ values, respectively.

Considering A_T and C_T as input variables to the computation of $[CO_2^*]$ (Eq. 4), C_T is a measured variable while the hydrogen ion concentration, $[H^+]$, needs to be estimated. The principal technique to achieve this, as applied in carbon cycle models and software tools like CO2SYS, involves iterative approaches such as the Newton-Raphson method (Lewis and Wallace, 1998; Orr et al., 1999; Follows et al., 2006). Terms given in the large bracket of Eq. (7) are computed for an initial guess of the pH, and for given thermodynamic constants (depending on T, S, and pressure), silicate, phosphate concentrations and total borate, fluoride and sulphate concentrations. The latter total concentrations are usually computed from relationships with salinity. The residual is analysed to derive a new estimate of the pH, from which in turn the various alkalinity components (large bracket) are recomputed. This process is repeated until the residual is sufficiently small and a consistent pH is derived.

$$
\begin{aligned}
\text{Residual} = {}& A_T \quad (7) \\
& - \left(\begin{array}{l} [HCO_3^-] + 2[CO_3^{2-}] + [B(OH)^-] + [OH^-] + [HPO_4^{2-}] \\ + 2[PO_4^{3-}] + [SiO(OH)_3^-] + [NH_3] + [HS^-] + ... \\ - [H^+] - [HSO_4^-] - [HF] - [H_3PO_4] - ... \end{array} \right)
\end{aligned}
$$

Software tools and carbon cycle models may differ on the treatment of the individual components of the bracketed term of Eq. (7). For example, [HS-] and [NH$_3$] are often omitted

since concentrations are usually negligible under oxic conditions and at seawater pH. Additional proton acceptors or donors, like organic acids and basis, are formally part of the alkalinity definition and indicated as ellipsis in Eqs. (1), (6) and (7), respectively. However, they usually have no representation in the software codes. Given that, in our thought experiment, the measurement of A_T contains a significant amount of organic acids, while the equations coded in the standard version of CO2SYS do not include these alkalinity components, an inaccurate pH will be derived. For the example of our thought experiment, computational errors (relative anomalies), $\Delta[H^+]$ and $\Delta[HCO_3^-]$, are shown in Fig. 4b. It is obvious that $\Delta f CO_{2(A_T, C_T)}$ scales with $\Delta[H^+]$. It is, hence, the inaccurate pH determination which gives rise to inaccurately computed $f CO_{2(A_T, C_T)}$.

For a fixed $f CO_2$ value (400 µatm) we explore the effect of potential DOM-A_T ranging between 0 and 50 µmol kg^{-1} on the computation of $f CO_2$. Leaving everything else unchanged, e.g., constant pK$_{Org}$, we apply CO2SYS-DOM to derive a second over-determined CO_2-system dataset with all four possible measurements, i.e., C_T, A_T, $f CO_2$ and pH. Applying pairs of these input data for the computation of $f CO_2$ with the standard version of CO2SYS, an almost linear and positive correlation is found between $\Delta f CO_{2(A_T, C_T)}$ and DOM-A_T (Fig. 5a), and a negative correlation of $\Delta f CO_{2(A_T, pH)}$ with DOM-A_T. Both relationships are very similar to those observed in the culture experiments between $\Delta f CO_2$ and the accumulated DOC (Fig. 5b). In particular, even quite high DOM-A_T the error $\Delta f CO_{2(A_T, pH)}$ remains small compared with the error $\Delta f CO_{2(A_T, C_T)}$.

The effect of DOM-alkalinity on the computation of $f CO_2$ from A_T and C_T input data also depends on the choice of the pK$_{Org}$ value of the organic acid/base system. We illustrate this quantitatively by assuming A_T of 2400 µmol kg^{-1}, DOM-A_T of 50 µmol kg^{-1}, and prescribing an $f CO_2$ of 400 µatm for a range of pK values between 4.6 and 8.5. Using CO2SYS-DOM we derive a third over-determined CO_2-system dataset and repeat the computation of $f CO_2$ from the three possible pairs of input data using the standard version of CO2SYS. As expected from theory $\Delta f CO_2$ decreases as the assumed pK approaches the mean pH of seawater (Fig. 6). For a wide range of pK values, however, the effect is independent of the choice of the pK$_{Org}$.

For consistency, we also checked whether uncertainties in thermodynamic constants contribute to the observed magnitude of $\Delta f CO_{2(A_T, C_T)}$. Applying several other published sets of thermodynamic constants, which may alternatively be chosen in CO2SYS, we find only small variations of $\Delta f CO_{2(A_T, C_T)}$ for different choices of K_1 and K_2 (Fig. 7).
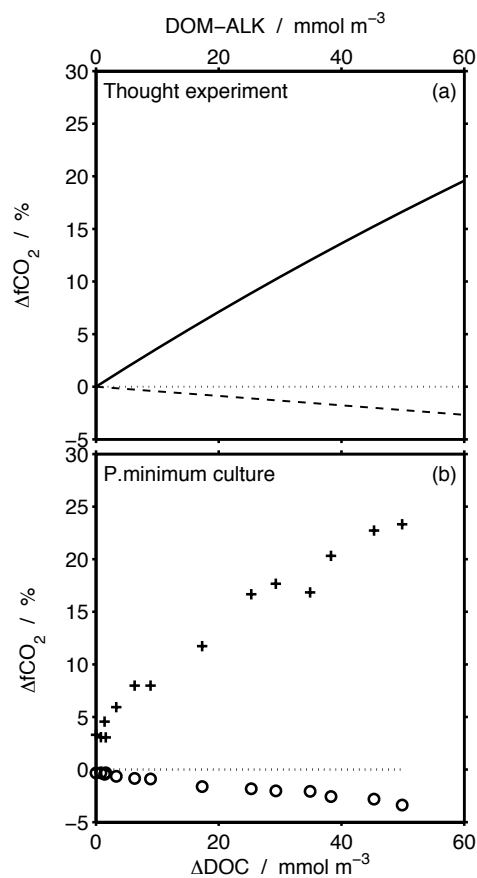
Fig. 5. $\Delta f\mathrm{CO}_{2(A_\mathrm{T},C_\mathrm{T})}$ (solid line, +), $\Delta f\mathrm{CO}_{2(A_\mathrm{T},\mathrm{pH})}$ (dashed line, o), and $\Delta f\mathrm{CO}_{2(C_\mathrm{T},\mathrm{pH})}$ (dotted lines) from **(a)** a thought experiment and numerical simulations (for details see Sect. 3.2) and **(b)** the *P. minimum* culture. X-axes is either the potential DOM-alkalinity ($\mu\mathrm{mol\,kg}^{-1}$) assumed in the numerical simulations **(a)** or **(b)** the measured DOC accumulation over the experiment ($\mu\mathrm{mol\,l}^{-1}$).

4   Discussion

4.1   How widespread is the issue of elevated $\Delta f\mathrm{CO}_{2(A_\mathrm{T},C_\mathrm{T})}$?

Data from a larger number of experimental ocean acidification studies have recently been archived in the Pangaea database (Nisumaa et al., 2010). This EPOCA/EUR-OCEANS data compilation also provides an overview of the dominantly used measurement protocols in recent ocean acidification experiments. About 90 % (49 %) of the papers from which data have been archived by Nisumaa and co-workers (see their Fig. 5) used A_T (C_T), respectively, as one of the measured variables of the carbonate system. That is, close to 50 % of the studies computed $f\mathrm{CO}_2$, carbonate ions or other variables of the CO_2-system from the pair of A_T and C_T. Only 17 % of the studies measured $p\mathrm{CO}_2$ directly and there is only a small number of experiments (about 5 %) during which the carbon dioxide system had been over de-
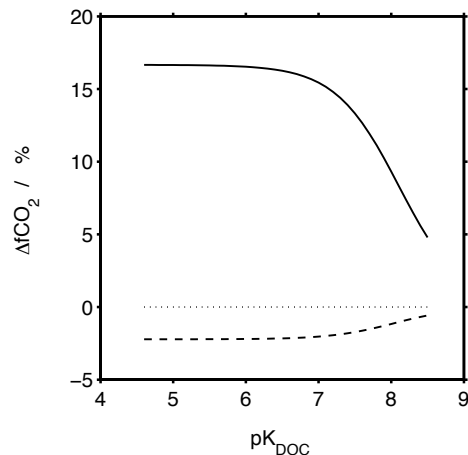

Fig. 6. $\Delta f\mathrm{CO}_{2(A_\mathrm{T},C_\mathrm{T})}$ (solid line), $\Delta f\mathrm{CO}_{2(A_\mathrm{T},\mathrm{pH})}$ (dashed line), and $\Delta f\mathrm{CO}_{2(C_\mathrm{T},\mathrm{pH})}$ (dotted lines) as a function of the choice of the pK of the organic acid/base system. We assume a potential DOM-alkalinity of $50\,\mu\mathrm{mol\,kg}^{-1}$, a true $f\mathrm{CO}_2$ of $400\,\mu\mathrm{atm}$, and standard conditions as explained in the text (Sect. 3.2).
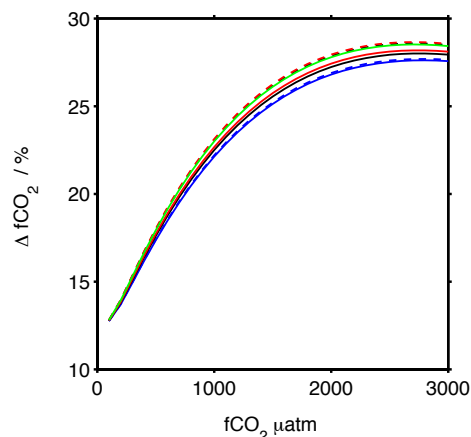

Fig. 7. $\Delta f\mathrm{CO}_{2(A_\mathrm{T},C_\mathrm{T})}$ as a function of the imposed $f\mathrm{CO}_2$ and for different choices of the carbonic acid dissociation constants as implemented in the standard version of CO2SYS (Roy et al. (1993) (blue, solid), Goyet and Poisson (1989) (blue, dashed), Hansson (1973a, b) refitted by Dickson and Millero (1987) (black, dashed), Mehrbach et al. (1973) refitted by Dickson and Millero (1987) (black, solid), Hansson and Mehrbach data refitted by Dickson and Millero (1987) (red, solid), Millero et al. (2006) (red, dashed), Lueker et al. (2000) (green, solid).

termined (Nisumaa et al., 2010). Also DOM measurements have been carried out in only a small subset of these experiments. It is, hence, very difficult to access the wider significance of our observations from the publicly available data.

Phytoplankton culture experiments described in detail by Camiro-Vargas et al. (2005) and Hernández-Ayon et al. (2007) provide an example which is even more extreme than the data presented here. These authors grew cultures

of *Rhodomonas* sp. in high nutrient (initial concentrations: $NO_3 = 1323\,\mu mol\,l^{-1}$, $PO_4 = 58.5\,\mu mol\,l^{-1}$) batch cultures. Using data of measured A_T, C_T, and pH from Hernández-Ayon et al. (2007), assuming a mean salinity of 35, and using $f CO_{2(C_T,pH)}$ as the reference, we compute values of $\Delta f CO_{2(A_T,C_T)}$ between about 30 % at the beginning of the experiment and up to 90 % about a week later. This huge $\Delta f CO_2$ is in agreement with a large observed difference between A_T measured using the derivative technique of Hernández-Ayón et al. (1999) and A_T computed from C_T and pH (up to $800\,\mu mol\,kg^{-1}$). Hernández-Ayon et al. (2007) suggest that this difference between measured and computed A_T, is indicative of a large accumulation of dissolved organic matter from phytoplankton growth stimulated by high initial concentrations of nitrate and phosphate. By the end of the experiment nitrate was depleted (Camiro-Vargas et al., 2005) and DOC had increased to $> 3000\,\mu mol\,l^{-1}$ (J. M. Hernández-Ayón, personal communication, 2010).

This finding points to an essential difference between open ocean datasets, which are characterised by a high degree of consistency between measured and computed $f CO_2$ (e.g., Lee et al., 2000) and typical experimental approaches often used in ocean acidification research. Experimental studies have often applied nutrient additions much higher than typical open-ocean seasonal nutrient variations. This consequently yields large phytoplankton blooms in experimental systems and in line with the closed-system nature of the experiments a marked production and temporal accumulation of fresh DOM (e.g., Goldmann et al., 1992; Norrman et al., 1995; Meon and Kirchman, 2001; Wetz and Wheeler, 2003; Wohlers et al., 2009). We compiled literature data describing net DOM accumulation observed in batch or mesocosm experiments. Given a considerable degree of variability, which most likely is related to dominant species, specific growth conditions and pre-incubation history, a general relationship between nitrate initially available and drawn down over the course of the experiments and net DOC accumulation is evident (Fig. 8). Seasonal DOC variations in surface waters of the open ocean are usually small, with about 3–6 $\mu mol\,kg^{-1}$ in subtropical waters near Bermuda (Hansell and Carlson, 2001) and 20–30 $\mu mol\,kg^{-1}$ in some high latitudes systems (e.g., Carlson et al., 1998). Most of the oceanic DOM is refractory. Importantly, experimental DOM accumulation is usually larger than the highest seasonal DOM variations observed in the ocean and up to two orders of magnitude larger than those reported from subtropical surface waters.

The fraction of freshly produced and accumulating DOM, which is in the form of organic acids or bases, and which may act as proton acceptors during alkalinity titration, is not known. Combining the experimental evidence of organic proton acceptors (i.e., ΔA_T; Hernandéz-Ayón et al., 2007; Muller and Bleie, 2008; Kim and Lee, 2009) with the widespread observation of large amounts of DOM accumulating in phytoplankton culture and bloom experiments
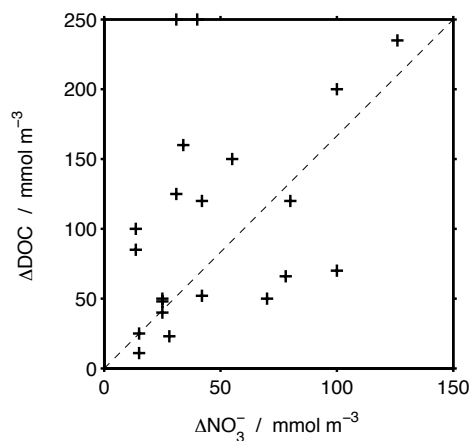

Fig. 8. DOC accumulation observed as a function of net drawdown of nitrate in phytoplankton culture and mesocosm experiments. Data were taken from the following references: Myklestad et al. (1989); Norrman et al. (1995); Biddanda and Benner (1997); Goldman et al. (1992); Meon and Kirchman (2001); Wetz and Wheeler (2003); Kragh and Søndergaard (2004); Engel et al. (2004); Schulz et al. (2008); Kim and Lee (2009); Wohlers et al. (2009).

(Fig. 8), however, we suggest that significant differences between measured $f CO_2$ and $f CO_2$ computed from A_T and C_T is not a singularity of our datasets, but likely as widespread as the DOM accumulation in such experiments itself. In the case where samples for alkalinity measurements are unfiltered, particle surfaces may additionally contribute to measured alkalinity (Kim et al., 2006) and provide an additional uncertainty to $f CO_2$ computed from A_T and C_T.

Because concentrations of DOM are also often elevated in coastal waters, compared with the open ocean (at times $> 200\,\mu mol\,C\,kg^{-1}$; e.g., Cauwet, 2002), coastal waters may occasionally be prone to the CO_2-computational issue shown here. This is due to additional sources, like river inflow (supplying humic substances to the sea), but also to larger autochthonous DOM production supported by higher nutrient levels and more intense plankton blooms. We are not aware of any published studies of CO_2-system over-determinations from coastal waters, that analyse the magnitude of $\Delta f CO_2$. One study, however, compared A_T measured using the derivative technique of Hernández-Ayón et al. (1999) and A_T computed from pH and C_T in the Gulf of California showing that measured and computed alkalinity may differ by $\Delta A_T = 50$ to $200\,\mu mol\,kg^{-1}$ (Hernández-Ayón et al., 2007). Extrapolating our simulations shown in Fig. 5a to such high DOM-alkalinities yields $\Delta f CO_{2(A_T,C_T)}$ of up to 50 % and $\Delta f CO_{2(A_T,pH)}$ of up to -10 %. So far no comprehensive analysis of ΔA_T and DOM is available for coastal waters. However, from the ΔA_T observed in Gulf of California waters by Hernández-Ayón et al. (2007), the elevated DOM concentrations found in coastal waters and

the elevated magnitude of phytoplankton blooms in coastal waters, partly by phytoplankton species known to release significant amounts of DOM (e.g., Cadée and Hegeman, 2002), we speculate that $\Delta f\mathrm{CO}_{2(A_\mathrm{T}, C_\mathrm{T})}$ in coastal waters may at times be significantly larger than in open ocean waters, and perhaps similar to what we observed in the experimental datasets of Kim and Lee (2009). From the strong seasonal variability of DOM concentrations in coastal waters (Williams, 1995; Suratman et al., 2009; Sintes et al., 2010) one can further speculate that $\Delta f\mathrm{CO}_{2(A_\mathrm{T}, C_\mathrm{T})}$ may show considerable seasonality as well. Further studies are needed.

4.2 Implications for global biogeochemical modelling in ocean acidification research

Global biogeochemical models are our major tools to extrapolate from small, experimental scales to the global ocean and into the future. It is via the use of such models that we may be able to extrapolate the experimentally observed sensitivities of, for example, calcium carbonate and organic matter production (e.g., Zondervan et al., 2001; Riebesell et al., 2008; Rost et al., 2008) or N_2-fixation rates (e.g., Barcelos e Ramos et al., 2007) to seawater $f\mathrm{CO}_2$ levels onto the global scale. Only global biogeochemical models will allow for a proper calculation of the net effect on e.g., atmospheric CO_2 of the various proposed biogeochemical responses and feedbacks to global warming, de-oxygenation and ocean acidification. Early model experiments aiming for first order quantifications of global impacts of ocean-acidification induced biogenic feedbacks on the carbon cycle (e.g., Heinze, 2004; Gehlen et al., 2007; Hofmann and Schellnhuber, 2009) based their parameterizations, e.g., of IC : OC ratios, on relatively small sets of experimental evidence yielding already a factor-of-four range in the simulated strength of the global CO_2-calcification feedback (Ridgwell et al., 2009). It is generally acknowledged that part of the difficulty of deriving robust parameterisations for biogeochemical impacts of ocean acidification arises from the fact that different species, strains and experimental conditions can lead to different responses for identical changes in the carbonate chemistry. Parameterisations of $CaCO_3$ production applied in global models have used either $f\mathrm{CO}_2$, $[\mathrm{CO}_3^{2-}]$, or the saturation state (Ω) as master variables (i.e., as x-axes) when putting together results from different experiments. The accuracy of these master variables of carbon dioxide chemistry has been taken for granted and not recognised as a potential source of uncertainty in the development of such parameterisations.

The majority of ocean acidification experiments studying the response of phytoplankton or whole pelagic ecosystems have reported A_T as one of two measured variables of the carbon dioxide system, and about 50 % of the reports compiled by Nisumaa et al. (2010) used the pair of A_T and C_T to compute other components of the CO_2 system. Given the results of our study, that the computation of master vari-

ables of the CO_2 system if based on A_T and C_T is uncertain due to the usually not quantified contribution of DOM to A_T, it is unclear how the available information from recent ocean acidification experiments can be safely used to develop such functional relationships. It is well possible that the response to ocean acidification found in some studies is overestimated when calculating $f\mathrm{CO}_2$ from A_T and C_T as suggested already by Hoppe et al. (2012), however, when results from a variety of studies with unknown reliability of $f\mathrm{CO}_2$ ($[\mathrm{CO}_3^{2-}]$, Ω) values and its time course are compiled together to derive some mean or general functional relationship, this relationship might simply be unconstrained.

5 Recommendations for ocean acidification experiments

The correlation of $\Delta f\mathrm{CO}_{2(A_\mathrm{T}, C_\mathrm{T})}$ with DOC accumulation inferred from measurements during an ocean acidification experiment points to the potential role of organic proton acceptors during alkalinity titration as one potential cause for elevated $\Delta f\mathrm{CO}_2$ presented in this study and elsewhere (Hernández-Ayón et al., 2007; Hoppe et al., 2012). Software tools regularly used to compute missing variables of the CO_2-system, like CO2SYS (Lewis and Wallace, 1998), which we used in our study, usually do not account for organic proton acceptors. Principally, DOM-alkalinity may be estimated using a back-titration technique (Cai et al., 1998). However, given the current lack of knowledge of the respective pK_Org an explicit treatment of DOM-alkalinity remains challenging. In that regard, the CO2SYS-DOM used in this study is a tool to demonstrate the potential problems when computing $f\mathrm{CO}_2$ from A_T and C_T, however, it is not recommended to be used as an operational tool in ocean acidification research.

Computing $f\mathrm{CO}_2$ from A_T and C_T instead of measuring it was a standard procedure in many ocean acidification experiments conducted so far (Nisumaa et al., 2010). We propose that computed $f\mathrm{CO}_2$ from these experiments published in the literature is uncertain. The same argument applies when computing carbonate ion concentrations ($[\mathrm{CO}_3^{2-}]$), saturation states (Ω), or pH from these experiments. Moreover, there is no easy way to quantify the magnitude of $\Delta f\mathrm{CO}_2$ ($\Delta p\mathrm{CO}_2$, $\Delta[\mathrm{CO}_3^{2-}]$) for the individual experiments since over determination of the carbon-dioxide system has been very rare in these experiments and DOM release has been measured only in a few studies. Amount, composition, and timing of DOM production and accumulation are all known to be variable. Culture and microcosm experiments for example have shown that a large increase in DOM is often associated with nutrient depletion (e.g., Berman and Holm-Hansen, 1974; Wetz and Wheeler, 2003), which might indicate that results from nutrient replete ocean acidification culture experiments are less affected and $\Delta f\mathrm{CO}_2$ is small here. However, other studies found species-specific response with high DOM release also

under nutrient replete conditions in some species and low release in others (e.g., Biddanda and Benner, 1997). Most importantly, some studies have suggested that DOC production and fate vary itself with pCO$_2$ (Engel et al., 2004), complicating the issue further.

In the recently published guide for the best practice for ocean acidification research and data reporting (Riebesell et al., 2010) the available measurement techniques for studies of the carbon dioxide system in seawater in ocean acidification experiments have been discussed and evaluated in detail. While for natural seawater the measurement of A_T and C_T is recommended (Dickson et al., 2007; Dickson, 2010), our analysis emphasises that in phytoplankton culture and bloom experiments the carbon dioxide may be better characterised by (spectrophotometrical) pH and appropriate (e.g., coulometric) C_T measurements (Dickson, 2010). Hoppe et al. (2012) proposed to combine pH and A_T measurements in order to best characterize the CO$_2$ system in ocean acidification experiments. To the extent that differences between the actual alkalinity and the common approach to estimate it (i.e., the seawater model of alkalinity in use) are responsible for differences between measured and computed fCO$_2$, the numerical experiments presented in this study (e.g., Fig. 5) indicate that the pair of measured pH and A_T will provide reasonable though not perfect estimates of fCO$_2$. We suggest that whenever possible experimentalists should over-determine the CO$_2$ system, adding appropriate pH or fCO$_2$ measurements to high quality A_T and C_T measurements. This additional effort will also help to better understand the extent of the DOM-alkalinity issue in general and with respect to already published experimental work, hopefully clarifying whether these results can still be used when deriving parameterisations for global simulations.

We base our concern on a relatively small number of experiments for which we have direct evidence for significant, large and temporally variable fCO$_2$ computation errors. Clearly there is a need for more such experimental studies, which should combine over-determination of the carbon dioxide system (i.e., high quality measurements of pH, C_T, A_T, fCO$_2$) and detailed determination of dissolved organic matter (e.g., Muller and Bleie, 2008), before the case can be settled. Given that amount and composition of DOM production and accumulation is highly variable, differs by species (phytoplankton group), growth rates and experimental conditions (e.g., Nagata, 2000 for an overview), we expect a high variability of ΔfCO$_{2(A_T, C_T)}$.

We conclude that difficulties in developing model parameterisations do not only arise from variations in the observed functional responses (of e.g., the IC:OC ratio, PIC production, POC production, etc.; i.e., values on the y-axes) but most likely also from uncertainties of the relevant values on the x-axes, i.e., the independent variables, often fCO$_2$, (pCO$_2$), pH, or [CO$_3^{2-}$] to which parameterisations refer.

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