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Combined effects of CO_2 and temperature on carbon uptake and partitioning by the marine diatoms *Thalassiosira weissflogii* and *Dactyliosolen fragilissimus*

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Abstract

Carbon uptake and partitioning of two globally abundant diatom species, *Thalassiosira weissflogii* and *Dactyliosolen fragilissimus*, was investigated in batch culture experiments under four conditions: ambient (15°C, 400 μatm), high CO_2 (15°C, 1000 μatm), high temperature (20°C, 400 μatm), and combined (20°C, 1000 μatm). The experiments were run from exponential growth into the stationary phase (six days after nitrogen depletion), allowing us to track biogeochemical dynamics analogous to bloom situations in the ocean. Elevated CO_2 had a fertilizing effect and enhanced uptake of dissolved inorganic carbon (DIC) by about 8% for *T. weissflogii* and by up to 39% for *D. fragilissimus*. This was also reflected in higher cell numbers, build-up of particulate and dissolved organic matter, and transparent exopolymer particles. The CO_2 effects were most prominent in the stationary phase when nitrogen was depleted and CO_2(aq) concentrations were low. This indicates that diatoms in the high CO_2 treatments could take up more DIC until CO_2 concentrations in seawater became so low that carbon limitation occurs. These results suggest that, contrary to common assumptions, diatoms could be highly sensitive to ongoing changes in oceanic carbonate chemistry, particularly under nutrient limitation. Warming from 15 to 20 °C had a stimulating effect on one species but acted as a stressor on the other species, highlighting the importance of species-specific physiological optima and temperature ranges in the response to ocean warming. Overall, these sensitivities to CO_2 and temperature could have profound impacts on diatoms blooms and the biological pump.

Over the past few centuries, atmospheric carbon dioxide (CO_2) concentrations increased from preindustrial levels of approximately 280 ppmv (parts per million volume) to around 400 ppmv in the year 2013 (IPCC 2007). This increase in atmospheric CO_2 leads to a global increase in temperatures and its subsequent uptake by the ocean results in a decrease in seawater pH, both occurring at a pace unprecedented in recent geological history (IPCC 2007; Doney et al. 2009). The reduction in seawater pH, a phenomenon commonly referred to as ocean acidification (Caldeira and Wickett 2003), is accompanied by changes in carbonate chemistry, leading to a strong decrease in carbonate ion (CO_3^{2-}) concentration, a slight increase in bicarbonate (HCO_3^-), and a strong increase in dissolved CO_2 and protons (H^+) (Zeebe and Wolf-Gladrow 2001). Both, changes in CO_2 and temperature affect the buffering capacity of seawater, which is usually given as the fractional change in CO_2 over the fractional change in dissolved inorganic carbon (DIC) (Revelle factor): $(\Delta CO_2/CO_2)/(\Delta DIC/DIC)$ (Egleston et al. 2010). At the same time, global average sea surface temperatures have already increased by 0.6°C since the beginning of the 20th century. Recent climate projections suggest an increase in global surface air temperatures of 1.1–6.4°C by the end of this century (relative to 1980–1999), thereby also leading to a further warming of the upper ocean (IPCC 2007). Both warming and ocean acidification are expected to affect marine ecosystems and carbon cycling in a variety of ways (for reviews see, e.g., Riebesell et al. 2009, Passow and Carlson 2012).

Temperature is a major environmental driver controlling metabolic rates, such as enzymatic reactions, thereby substantially affecting phytoplankton physiology (Duarte 2007; Boyd et al. 2013). Furthermore, it has been found that ocean warming might lead to a decrease in cell size (Moran et al. 2010), shifts in phenology (Sommer and Lengfellner 2008), and alterations in carbon cycling by phytoplankton (Wohlers et al. 2009; Taucher et al. 2012). Concurrently, ocean acidification is expected to influence marine phytoplankton

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either through a decrease in pH (and the corresponding increase in H^+ ions) or the shift in carbonate speciation, e.g., the relative availability of CO_2 and HCO_3^- for carbon assimilation and photosynthesis. Over the last few years, an increasing number of studies investigated effects of ocean acidification on various functional groups of phytoplankton, showing, for example, a marked influence of carbonate chemistry on growth and calcification in coccolithophores (Riebesell et al. 2000; Langer et al. 2006; Iglesias-Rodriguez et al. 2008) and nitrogen fixation in cyanobacteria (Hutchins et al. 2009). Furthermore, it has been demonstrated that simultaneous changes in temperature and CO_2 can have interactive effects on growth rates, photosynthesis, and elemental composition of various marine phytoplankton (Feng et al. 2008; Fu et al. 2007, 2008).

Relatively few studies exist about the effects of ocean acidification on diatoms, which account for 40% of total primary production in the ocean (Nelson et al. 1995), frequently form massive blooms, and are commonly assumed to dominate export production (Lampitt 1985; Treguer et al. 1995; Buesseler 1998), making them major players in marine biogeochemical cycling and sequestration of carbon to the deep ocean (Sarthou et al. 2005; Boyd et al. 2010).

Diatoms, like most other noncalcifying phytoplankton, are assumed to be affected by ocean acidification primarily through changes in the availability of dissolved CO_2 as a substrate for photosynthesis (Rost et al. 2008). Concentrations of CO_2 in the surface ocean are relatively low compared to other forms of inorganic carbon, especially HCO_3^-. Yet, the primary carboxylating enzyme used in photosynthesis, RubisCO, is restricted to CO_2 for fixation of inorganic carbon, even though its substrate affinity for CO_2 is rather low. Therefore, in addition to passive uptake of CO_2 via diffusion, most phytoplankton operate a carbon concentrating mechanism (CCM) to enhance their intracellular CO_2 relative to external concentrations (for a recent review see Reinfelder 2011). Although the exact functioning of the CCM varies greatly among and within the dominant groups of marine phytoplankton, it generally involves active uptake of CO_2 and/or HCO_3^- into the cell interior, as well as external or intracellular activity of the enzyme carbonic anhydrase, which accelerates the otherwise slow conversion between HCO_3^- and CO_2. For diatoms, there is evidence that their CCM is highly regulated and efficient with respect to changes in CO_2 (Rost et al. 2003; Trimborn et al. 2009). Correspondingly, most studies find little or no change in photosynthetic rates of diatoms under elevated pCO_2, suggesting that carbon assimilation is essentially saturated under present-day CO_2 levels and thus unlikely to be affected by ocean acidification (Burkhardt et al. 1999; Rost et al. 2008; Beardall et al. 2009). However, this is in sharp contrast to the observed effect of elevated CO_2 on inorganic carbon uptake by diatoms in natural plankton communities (Riebesell et al. 2007; Tortell et al. 2008).

This controversy might result from the fact that laboratory-based studies usually do not take into account bloom dynamics and the postbloom physiological states of diatoms. However, exponential growth under nutrient-replete conditions and constant carbonate chemistry are not necessarily reflective of behavior during a natural bloom, where nutrient limitation and substantial shifts in the carbonate system occur. Considering the energetic and nutrient costs associated with phytoplankton CCMs, it seems likely that potential effects of environmental factors such as temperature and CO_2 on the physiological performance of phytoplankton become more pronounced in the stationary phase when nutrients are depleted and resource allocation becomes critical (Giordano et al. 2005; Hopkinson et al. 2011). Furthermore, it has long been recognized that a substantial portion of carbon uptake can occur after nutrient depletion and lead to a notable decoupling of carbon and nitrogen uptake, a phenomenon commonly referred to as "carbon overconsumption" (Sambrotto et al. 1993; Toggweiler 1993). These dynamics during the declining phase of a bloom are particularly important for export production (Lampitt 1985) and results from previous mesocosm experiments with natural, diatom-dominated plankton communities indicate that carbon overconsumption is affected by both temperature (Taucher et al. 2012) and CO_2 (Riebesell et al. 2007). However, carbon uptake and its allocation between the particulate and dissolved phase by primary producers on the one side, and heterotrophic consumption of this organic matter on the other side, operate simultaneously and are closely intertwined under natural or seminatural conditions in such studies, making it difficult to disentangle the underlying mechanisms.

The aim of this study is to help resolve this controversy and specifically investigate the effects of warming and increased pCO_2 on carbon uptake by two marine diatom species, *Thalassiosira weissflogii* and *Dactyliosolen fragilissimus*, with particular focus on dynamics after N-depletion, as well as the partitioning of this carbon between the particulate and dissolved phase.

Methods

Pre-experiments to determine the temperature range of the diatoms

Pre-experiments (referred to as temperature gradient experiment throughout) were carried out to identify the temperature range and optimum of two diatom species, by setting up a temperature gradient from 10°C to 22.5°C.

The aim was to choose two species that co-occur in nature but have different temperature optima, so that warming in the main experiment had a stimulating effect on one species, but acted as a stressor on the other species, pushing it beyond its temperature optimum. Thereby, a range of potential responses to future ocean conditions, i.e., warming in

combination with increased CO_2, and possible synergistic effects could be investigated in the main experiments. Ideally, we would have carried out the main experiments with temperatures below and above the optimum for both species. However, this was not feasible due to logistical reasons.

To identify temperature range and optimum of the diatom species, cultures were grown in gas-tight polycarbonate bottles (one liter) at ambient pCO_2 (400 μatm) under six temperatures (10.0°C, 12.5°C, 15.0°C, 17.5°C, 20.0°C, and 22.5°C) that were established with the help of temperature controlled water baths in two climate-controlled rooms. Prior to incubation at target temperatures, both diatom species were preacclimated to 12.5°C and 20°C for the lower and upper three temperature levels, respectively, for one week. Light supply and nutrient availability were identical to those of the main experiment (see below). Daily measurements were restricted to pH, NO_3^-, and in vivo fluorescence (F_T) due to the small volume of the incubation bottles (see Sampling and measurements section for details on measurement methods). Additional samples for particulate and dissolved organic matter (DOM) were taken on the first day of the experiment (t_0), the day the respective cultures reached NO_3 depletion (t_{N0}), and four days after NO_3 depletion (t_{N4}). Optimum temperature was defined as that supporting maximal growth rates as determined by daily changes in fluorescence.

Experimental setup

Phytoplankton strains

All experiments presented here were conducted with monoclonal cell cultures of two globally abundant diatom species (Leblanc et al. 2012), using the single-celled and rather small species *T. weissflogii* (10–20 μm cell diameter, strain CCMP 1336), and the larger, chain-forming species *D. fragilissimus* (50–100 μm cell diameter, isolated from the North Sea in September 2012, now listed as strain RCC3389).

Treatments: Combination of temperature and CO_2

For each diatom species, eight gas-tight polyethylene bags were set up in duplicate in a combination of two temperatures and two pCO_2 levels, with temperatures of 15°C and 20°C, initial partial pressure levels of CO_2 of 400 μatm and 1000 μatm. In the following, these treatments will be referred to as: "ambient" (15°C and 400 μatm), "high CO_2" (15°C and 1000 μatm), "warming" (20°C and 400 μatm), and "combined" (20°C and 1000 μatm), respectively. Light supply was set to a photon flux density of $\sim$ 100 μmol m^{-2} s^{-1} and a light/dark cycle of 14/10 h in all treatments.

Materials, seawater, and nutrients

Collapsible bags (100 × 40 cm) constructed out of six millimeter polyethylene were used as incubation vessels for these experiments and allowed us to incubate the cultures without the presence of a headspace. The bags were suspended in large water baths to maintain temperature and minimize gas exchange through the bag walls. The initial volume of the bags was 20 L. As volume was removed from the incubation the bags were rolled up to minimize the surface area to volume ratio. The bags were sterilized and dissolved organic carbon (DOC) leaching minimized by soaking in 10% HCl for at least 48 h prior to the experiment. Preliminary experiments showed no significant change in DIC or DOC over a period of 10 d in cleaned bags containing only sterile artificial seawater (ASW).

The experiment with *T. weissflogii* was conducted with ASW, following Kester et al. (1967). Since, *D. fragilissimus* did not grow well in ASW, experiments with this species were carried out in natural seawater (NSW) from the Santa Barbara Channel, which was collected in late April 2013 and subsequently filtered through 1.2 μm and 0.2 μm filters. Media was not autoclaved to avoid perturbation of the carbonate chemistry beyond the range found in NSW. Both culture media (ASW and NSW) were instead treated with a UV lamp (50 Watt) for 30 min before filling into the bags to minimize DOC and bacterial contamination. Preliminary experiments showed that such a UV-treatment reduced bacterial production (BP) below detection limit for at least a week.

ASW and NSW were enriched with inorganic nutrients in a modified version of f/2 medium (Guillard and Ryther 1962), with concentrations calculated in such a way that the diatom cultures became nitrate limited. Initial concentrations of NO_3^- were identical in both experiments, amounting to 15 μmol L^{-1}. Starting concentrations for PO_4^{3-} were 6 μmol L^{-1} and 8 μmol L^{-1}, and 16 μmol L^{-1} and 50 μmol L^{-1} for $Si(OH)_4$ in the experiments with *T. weissflogii* and *D. fragilissimus*, respectively. Trace metals and vitamins were added at f/8 levels. The higher concentrations of PO_4^{3-} and $Si(OH)_4$ in the experiment with *D. fragilissimus* were based on preliminary experiments, which suggested a possibly higher Si and P demand compared to *T. weissflogii*. Furthermore, the ASW was further enriched with 2 ml L^{-1} of filtered (0.2 μm) and UV-blasted NSW to provide possible micronutrients that are not included in the recipe of Guillard and Ryther (1962).

Carbonate chemistry manipulation

Carbonate chemistry in our experiments was manipulated following the "closed system approach", i.e., adjusting pCO_2 at constant total alkalinity (TA) through combined addition of inorganic carbon, and acid or base to an incubation container without headspace (Rost et al. 2008). Calculations for the carbonate system manipulations were carried out with the program CO2SYS (Lewis and Wallace 1998), using the dissociation constants for carbonic acid as refitted by Dickson and Millero (1987) and taking into account nutrient additions for TA adjustment as well as respective temperatures for target pCO_2 levels.

For the experiments with ASW, the carbonate system was adjusted to target levels of pCO_2 and TA of 2350 μmol kg^{-1} through additions of 1 M solutions of HCO_3^- as DIC and NaOH/HCl to keep TA constant.

The carbonate system in the experiments with NSW had to be adjusted differently. The collected water had a pH of 7.76 and TA of $\sim$ 2290 μmol kg^{-1}. From that, we calculated the necessary amount of HCO_3^- addition to set the desired pCO_2 levels of 400 μatm and 1000 μatm for the respective temperatures (15°C and 20°C). To simulate the carbonate system dynamics of a natural plankton bloom, pCO_2 was adjusted only initially, and was then allowed to change freely, as driven by biological dynamics. Measurements confirmed that the desired initial experimental conditions were within $\sim$ 0.02 pH units (or $\sim$ 20 μatm pCO_2) of target conditions.

Acclimatization phase

Prior to the experiments, the diatom cultures were acclimatized to the respective target conditions of temperature and pCO_2 for at least a week ($\sim$ 5–6 generations). During this acclimatization phase, the cultures were grown semicontinuously in gas-tight polycarbonate bottles (one liter). When pCO_2 dropped below 200 μatm (for the 400 μatm treatment) or 700 μatm (for the 1000 μatm treatment), the cultures were diluted with fresh medium and pCO_2 was readjusted to target conditions by combined additions of HCO_3^- and HCl or NaOH. After acclimatization, diatom cultures were inoculated at a density of 500 cells mL^{-1} (*T. weissflogii*) and 200 cells ml^{-1} (*D. fragilissimus*) to the respective experimental treatments.

Sampling and measurements

Sampling was carried out daily, immediately at the end of each light cycle. This maximized the comparability of samples between treatments and replicates, as all the cultures were sampled in the same physiological state. The bags were mixed gently prior to sampling to obtain representative water samples. Samples for pH and DOC were taken directly from the bag (gravity-filtration through in-line GF/F filter). For all other parameters, subsamples were collected in one liter polycarbonate bottles and later subdivided for various analyses. Since a major focus of our culture experiments was to determine carbon overconsumption and dynamics in the stationary phase, we continued sampling for (at least) six days after NO_3 depletion.

pH was measured spectrophotometrically (Thermo Scientific Genesys 105 VIS Spectrophotometer with a SPG 1A air-cooled single cell Peltier element) at 25°C following Dickson et al. (2007), using *m*-cresol as an indicator dye. For maximum precision and accuracy, the dye was calibrated against certified reference material (A. Dickson, La Jolla, California).

DIC was measured on a nondispersive infrared analyzer following Bandstra et al. (2006). Samples were filtered through GF/F filters and stored in $\sim$ 400 mL borosilicate bottles, sealed gas-tight, and stored at $\sim$ 5°C until analysis. Additional carbonate system variables were calculated from measured DIC and pH with the program CO2SYS (Lewis and Wallace 1998), using the dissociation constants for carbonic acid as refitted by Dickson and Millero (1987).

Samples for particulate organic carbon and nitrogen (POC and PON) were filtered onto precombusted (five hours at 450°C) GF/F filters, rinsed with ASW immediately after filtering of samples in order to avoid accumulation of DOM on the filters, dried at $\sim$ 60°C for approximately 24 h and stored under vacuum in a desiccator until analysis. Afterward, they were analyzed on a CHN organic elemental analyzer (model CEC 440HA, Control Equipment Corp., now Exeter Analytical).

Samples for DOC were gravity-filtered through precombusted GF/F filters, with the filtrate collected in combusted (five hours at 450°C) glass vials and frozen (at −20°C) until analysis. DOC was quantified via high-temperature combustion on a Shimadzu TOC-V as in Carlson et al. (2010), but with glucose calibration standards and Santa Barbara Channel reference standards that were routinely verified against DOM consensus reference water (Hansell 2005), for a resolution of 1.5 μmol L^{-1}. Total dissolved nitrogen (TDN) was measured using the TOC-V (Farmer and Hansell 2007). Dissolved organic nitrogen (DON) was calculated by subtracting dissolved inorganic nitrogen (measured as described below) from TDN.

Samples for transparent exopolymer particles (TEP) were filtered onto 0.4 μm polycarbonate filters and subsequently stained with Alcian Blue following the procedure of Passow and Alldredge (1995). TEP concentration was measured colorimetrically on a spectrophotometer (Thermo Scientific Genesys 105 VIS Spetrophotometer) through absorption at 787 nm. The dye solution had previously been calibrated using Gum Xanthan and the concentration of TEP is expressed as μg Gum Xanthan equivalents per liter (μg GXeq L^{-1}). Conversion of TEP to carbon was not applied here, as conversion factors are highly species-specific and not available for *D. fragillisimus* to date (Engel and Passow 2001).

Samples for dissolved inorganic nitrate, nitrite, phosphate, and silicate were sterile-filtered through 0.2 μm surfactant-free cellulose acetate filters, collected in 20-mL HDPE vials, and stored at −20°C until analysis. Measurement was carried out on a flow injection analyzer (QuikChem 8000, Lachat Instruments, Zellweger Analytics).

BP was determined by the ^{3}H-leucine incorporation (^{3}H-leu, SA = 54.1 Ci mmo1^{-1}, Perkin Elmer, Boston, Massachusetts) method (Kirchman et al. 1986), using the microcentrifuge method (Smith and Azam 1992), modified by decanting supernatant rather than aspirating. Samples were taken on the first day of the experiment, as well as one and six days after N-depletion. The samples were then dark-incubated for 1.5–3 h and radioactivity was analyzed on a Beckman LS 6500 Liquid Scintillation Counter. All incubations were

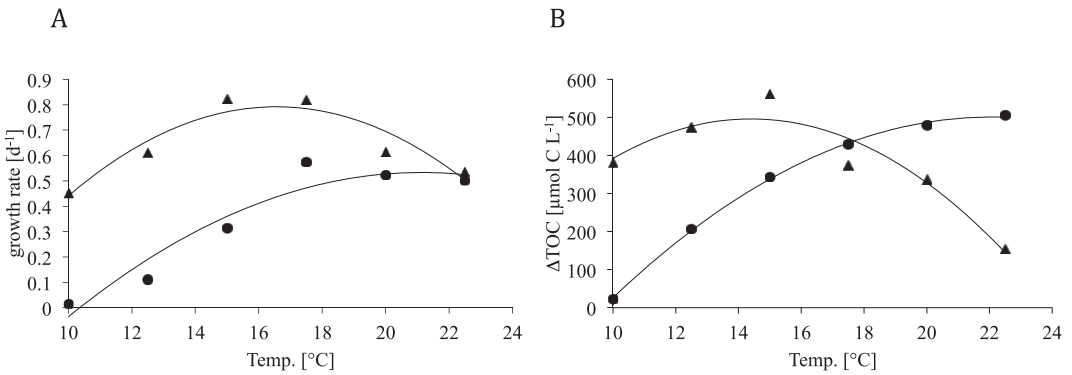

Fig. 1. Effect of temperature on diatom growth and carbon cycling. Shown are (A) growth rate (d^{-1}) under nutrient-replete conditions (calculated from fluorescence), (B) cumulative build-up of total organic carbon (μmol C L^{-1}) from onset of the experiment until day 4 after N-depletion for *T. weissflogii* (circles) and *D. fragilissimus* (triangles). Solid lines denote significant relationship of temperature and the respective parameter detected by polynomial regression ($p < 0.05$, $n = 6$).

carried out in triplicate. To convert the incorporation of ^{3}H-leucine into carbon production (μg C L^{-1} h^{-1}), a theoretical conversion factor of 1.5 kg C mol^{-1} leucine was used (Simon and Azam 1989).

Samples for cell concentrations were fixed with buffered formalin (40%) at a final concentration of 1% and counted microscopically using a hemocytometer.

In vivo dark-adapted chlorophyll fluorescence (F_T) was measured using an AquaPen-C fluorometer (Photon Systems Instruments, Czech Republic). Samples were kept in the dark for at least 10 min before the measurement.

Statistical analysis

For the temperature gradient experiment, a polynomial regression was fitted to the data to identify the temperature optimum of the two diatoms species ($n = 6$). The analysis was based on growth rate as calculated from measured fluorescence during nutrient-replete growth as a function of temperature.

In the main experiments, we applied a fully crossed two-factorial design with temperature and pCO_2 as the fixed factors and two treatment levels, respectively. Each treatment was set up in duplicates ($n = 2$). All results below are given as the average value of replicates ± standard error, which corresponds to the range of replicates for $n = 2$.

Statistical analysis for the detection of treatment effects was carried out with a permutational analysis of variance (PERMANOVA), because assumptions of normality and homogeneity of variances were not met for all analyzed data (Anderson 2001). For the analysis, "Euclidean distance" matrices with 9999 permutations were used, and pairwise (post hoc) tests were performed in case a significant effect ($p < 0.05$) was detected. PERMANOVA was applied for maximum values of net DIC uptake and net build-up of POC and DOC, as well as their respective C : N ratios, relative to day 0 for each replicate. For cell concentrations and TEP, the average concentration in the stationary phase after nitrogen depletion was used, as measurements were highly variable in the experiment with *D. fragilissimus* after N-depletion.

Results

Temperature range and optimum of the two diatom species

The temperature gradient experiment revealed a significant effect of temperature on growth rate of both diatom species (Fig. 1A). *T. weissflogii* displayed a positive response over the entire range of temperatures from 10°C to 22.5°C, with an optimum at ~ 20°C. In contrast, growth rate and TOC build-up by *D. fragilissimus* increased over the range from 10°C to 15°C but decreased beyond that, suggesting an optimum temperature of ~ 15°C. Net build-up of organic carbon (TOC = POC + DOC) including the stationary phase (until four days after N-depletion) displayed a similar response to temperature (Fig. 1B).

Main experiments

The development of the diatom cultures in both experiments was characterized by a rapid decline in dissolved inorganic nutrients, drawdown of DIC, decrease in pCO_2 and dissolved CO_2, and build-up of particulate (POM) and DOM (Figs. 2-4).

Growth and nutrient uptake

Initial concentrations of NO_3^- were 15 μmol L^{-1} in both experiments. In the experiment with *T. weissflogii*, NO_3^- was depleted faster at higher temperatures, reaching concentrations close to zero at t3 (20°C) and t5 (15°C) in both pCO_2 treatments (indicated in Figs. 2, 3). In the experiment with *D. fragilissimus*, NO_3 depletion occurred on t3 in all temperature and pCO_2 treatments. PO_4^{3-} dropped from initial concentrations of 6 μmol L^{-1} and 9 μmol L^{-1} in the experiments with *T. weissflogii* and *D. fragilissimus*, respectively, but always remained > 2 μmol L^{-1} and thus well above

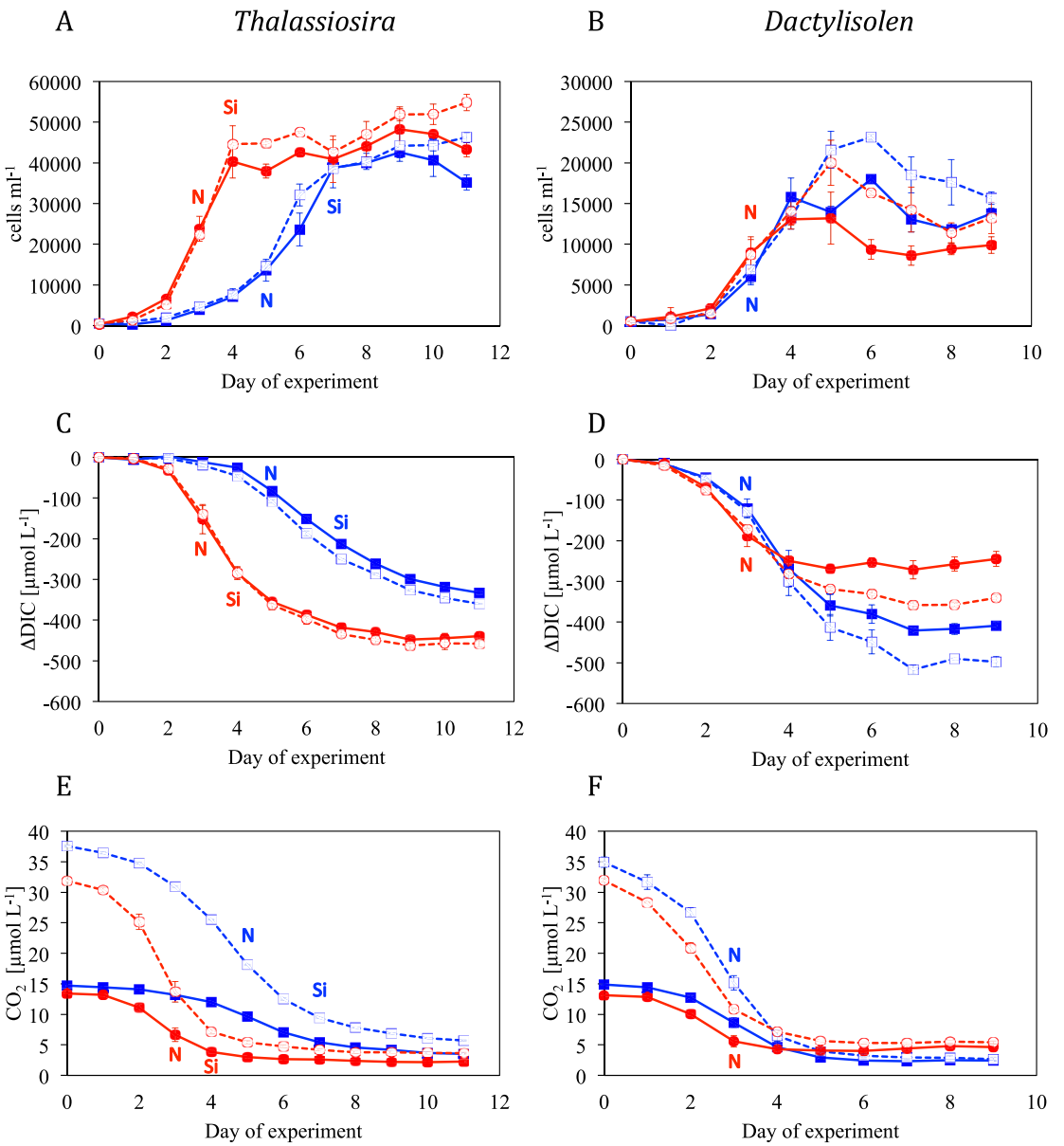

Fig. 2. Cell numbers and carbonate system variables in the experiment with *T. weissflogii* (left) and *D. fragilissimus* (right). Shown are temporal development of (A, B) cell concentrations, (C, D) DIC uptake relative to initial values (μmol L^{-1}) and (E, F) concentrations of dissolved CO_2 (μmol L^{-1}) at 15°C (blue squares) and 20°C (red circles), and in the ambient CO_2 (solid line, closed symbols) and high CO_2 treatments (dashed line, open symbols). Vertical error bars denote range of replicates within each treatment ($n = 2$). Colored letters denote day when cultures reached depletion of inorganic nitrogen (N) and silica (Si) in the 15°C (blue) and 20°C (red) treatments.

depletion. While Si remained replete in the *D. fragilissimus* experiment (~ 40 μmol $Si(OH)_4$ L^{-1}), it reached very low concentrations (< 2 μmol $Si(OH)_4$ L^{-1}) in the *T. weissflogii* experiment on t4 and t7 at 20°C and 15°C in both pCO_2 treatments, respectively. No effect of pCO_2 on the rate and magnitude of nutrient uptake could be detected in either of the experiments.

The drawdown of nutrients was reflected in rapid growth and an increase in cell concentrations in both experiments (Fig. 2A,B). In the *T. weissflogii* experiment, cell numbers increased from initial values of ~ 500 to average concentrations of 33,491 ± 1646 (ambient), 39,717 ± 1985 (warming), 37,218 ± 2112 (high CO_2) and 42,950 ± 3968 (combined) cells mL^{-1} in the stationary phase. In the experiment with *D. fragilissimus*, cell concentrations reached average values of 13,009 ± 40 (ambient), 10,149 ± 387 (warming), 16,477 ± 137 (high CO_2), and 13,991 ± 1009 (combined) cells mL^{-1} in the stationary phase. Thus, temperature had a significant effect on average cell concentrations in both experiments. High initial CO_2 resulted in elevated cell numbers in both diatom

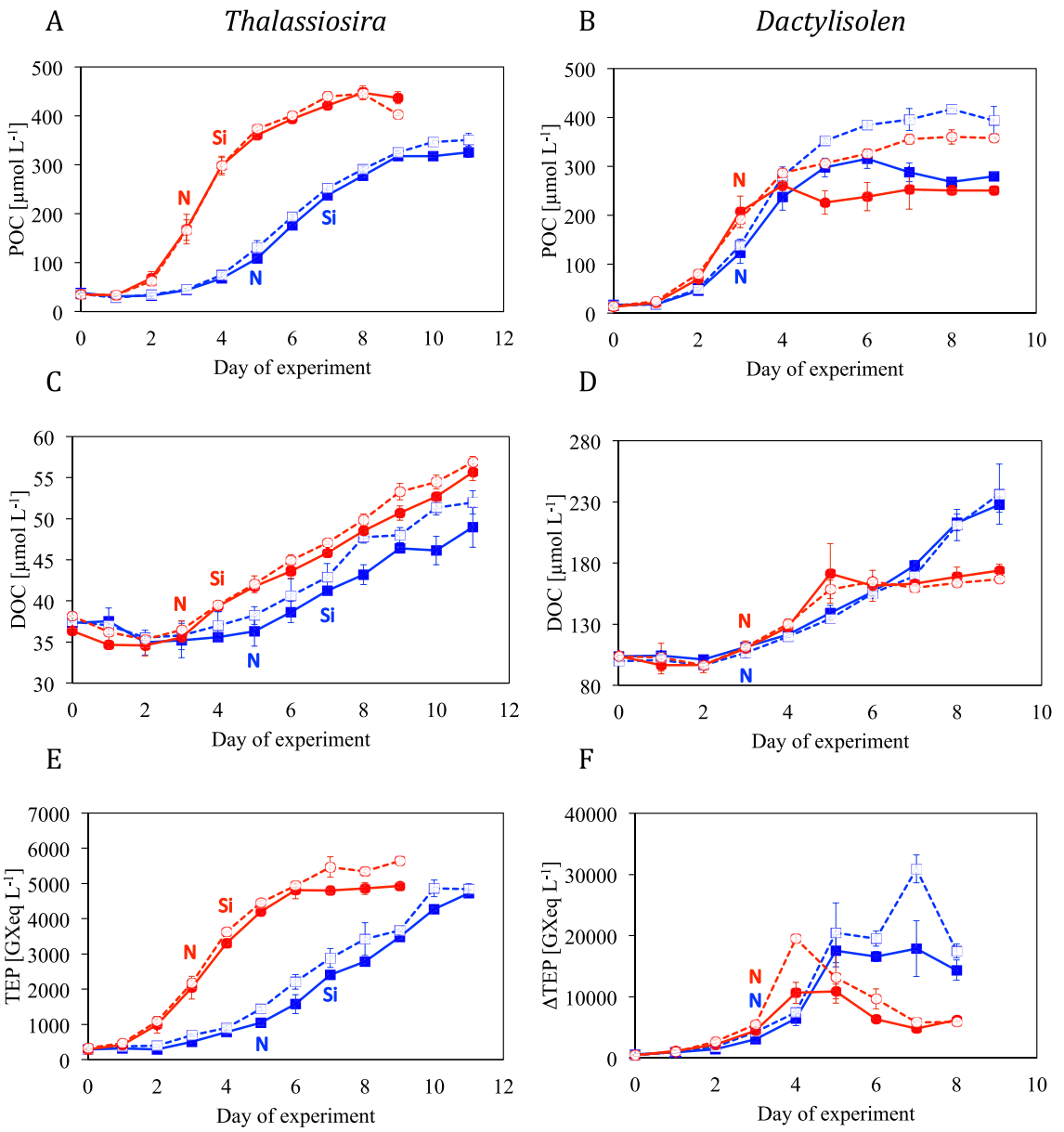

Fig. 3. Carbon partitioning by *T. weissflogii* (left) and *D. fragilissimus* (right). Shown are temporal development of measured (A, B) POC and (C, D) DOC concentrations (μmol L^{-1}), and (E, F) TEP concentrations (μg GXeq L^{-1}) in the experiment with *T. weissflogii* (left) and *D. fragilissimus* (right). Style and color-coding as in Fig. 2.

species, however, the effect was only statistically significant for *D. fragilissimus* and not for *T. weissflogii* (Tables 1, 2).

DIC uptake and build-up of POC, DOC, and TEP

DIC uptake showed a significant response to temperature and pCO_2 in both experiments (Fig. 2C,D; Tables 1, 2). Maximum uptake of DIC by *T. weissflogii* amounted to 333.2 ± 3.2 (ambient) μmol C L^{-1}, 360.2 ± 0.8 (high CO_2) μmol C L^{-1}, 448.1 ± 9.0 (warming) μmol C L^{-1}, and 462.7 ± 9.6 (combined) μmol C L^{-1}. In the *D. fragilissimus* experiment, maximum drawdown of DIC reached 420.7 ± 8.4 (ambient) μmol C L^{-1}, 516.9 ± 10.7 (high CO_2) μmol C L^{-1}, 271.6 ± 11.6 (warming) μmol C L^{-1}, and 358.4 ± 9.1 (combined) μmol C L^{-1}. Elevated pCO_2 enhanced biologically mediated drawdown of DIC by both species, even though the magnitude of the effect was much larger for *D. fragilissimus* (increase by 22–39%) than for *T. weissflogii* (increase by 3–8%).

The temperature increase from 15°C to 20°C significantly accelerated and enhanced DIC uptake by *T. weissflogii*. This response was more complex in the experiment with *D. fragilissimus*, where elevated temperatures led to a significant

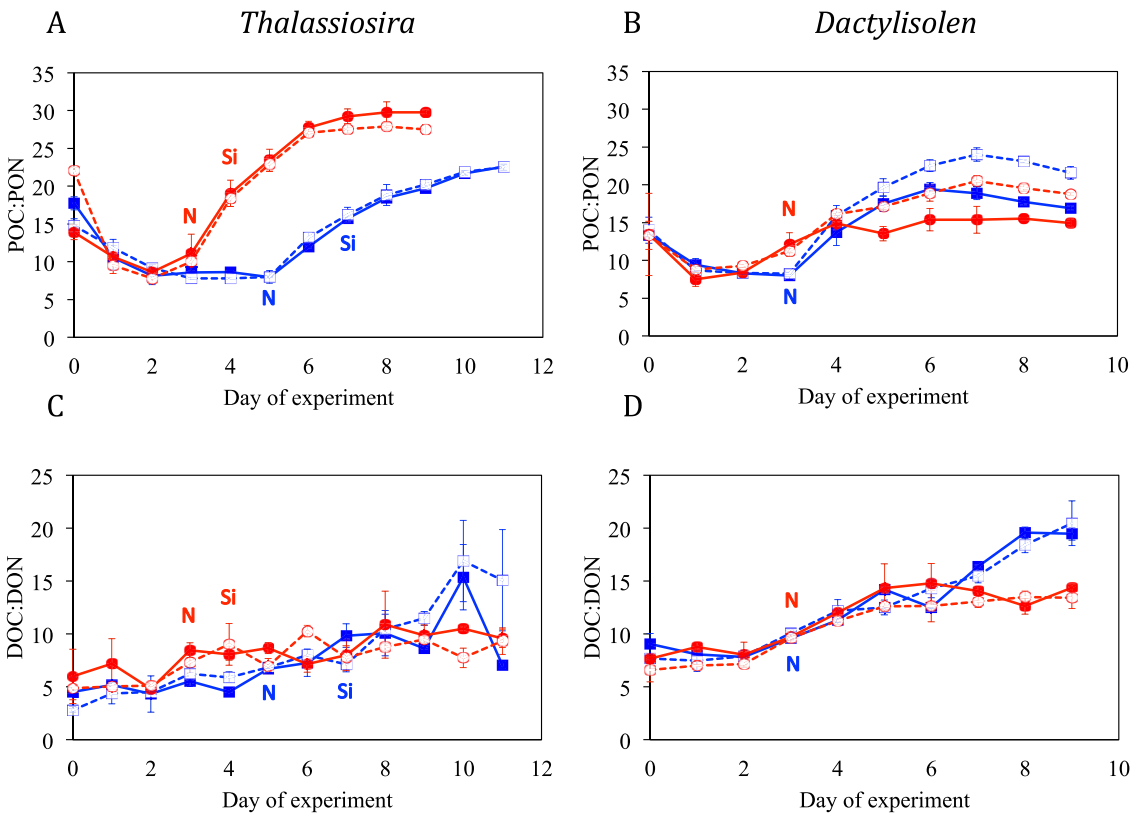

Fig. 4. Temporal development of C : N in (A, B) particulate and (C, D) dissolved organic matter in the experiment with *T. weissflogii* (left) and *D. fragilissimus* (right). Style and color-coding as in Fig. 2.

increase ($p < 0.05$) in DIC drawdown under nutrient-replete conditions, but had a negative effect once nitrogen was depleted (Fig. 2B).

The drawdown of DIC was reflected in a concomitant build-up of POC (Fig. 3A,B). In the *T. weissflogii* experiment, maximum POC build-up amounted to 288.7 ± 8.3 (ambient) μmol C L^{-1}, 314.7 ± 13.0 (high CO_2) μmol C L^{-1}, 412.0 ± 14.0 (warming) μmol C L^{-1}, and 418.1 ± 3.5 (combined) μmol C L^{-1}. POC build-up was enhanced at higher temperatures, whereas higher initial pCO_2 levels had no statistically significant effect (Fig. 3A; Table 1). Build-up of POC by *D. fragilissimus* reached maximum values of 298.3 ± 19.5 (ambient) μmol C L^{-1}, 402.5 ± 2.1 (high CO_2) μmol C L^{-1}, 248.7 ± 10.9 (warming) μmol C L^{-1}, and 346.0 ± 4.3 (combined) μmol C L^{-1}. Thus, both temperature and pCO_2 had a significant effect on maximum POC build-up, resulting in a decrease at temperatures beyond its optimum, but an increase at elevated pCO_2 (Fig. 3B; Table 2). Similar to DIC uptake, temperature had a significant positive effect on POC build-up before N-depletion ($p < 0.05$), but a negative effect afterward.

At the same time, accumulation of DOC was observed over the course of both experiments (Fig. 3C,D). In the *T. weissflogii* experiment, maximum DOC build-up amounted to 11.6 ± 2.4 (ambient) μmol C L^{-1}, 14.5 ± 1.4 (high CO_2) μmol C L^{-1}, 19.3 ± 1.0 (warming) μmol C L^{-1}, and 18.7 ± 0.7 (combined) μmol C L^{-1}. Maximum accumulation of DOC was much greater in the *D. fragilissmus* experiment, reaching 123.9 ± 6.2 (ambient) μmol C L^{-1}, 136.4 ± 24.6 (high CO_2) μmol C L^{-1}, 70.0 ± 8.3 (warming) μmol C L^{-1}, and 63.2 ± 0.3 (combined) μmol C L^{-1}. Consequently, elevated temperatures had a significant effect on DOC production by both species, resulting in an increase by *T. weissflogii*, but a decrease by *D. fragilissimus*. CO_2 did not have a statistically significant effect on DOC build-up by either of the species (Tables 1, 2).

A notable accumulation of TEP accompanied the accumulation of biomass in both experiments. In the *T. weissflogii* experiment, average TEP accumulation in the stationary phase after N-depletion amounted to $\sim 2900 \pm 80$ (ambient) μg GXeq L^{-1}, 3330 ± 180 (high CO_2) μg GXeq L^{-1}, 4130 ± 50 (warming) μg GXeq L^{-1}, and 4520 ± 50 (combined) μg GXeq L^{-1}. Thus, increases in temperature and CO_2 both significantly enhanced TEP accumulation (Fig. 3E; Table 1). The production of TEP by *D. fragilissimus* was generally much higher than that of *T. weissflogii*, reaching average concentrations of $\sim 11{,}800 \pm 1520$ (ambient) μg GXeq L^{-1}, $15{,}660 \pm 1060$ (high CO_2) μg GXeq L^{-1}, 7940 ± 510

Table 1. Effects of temperature and CO_2 on biogeochemical parameters in the experiment with *T. weissflogii*. Shown are maximum net uptake of DIC, maximum net build-up of POC, and DOC (μmol C L^{-1}), maximum ratios of DIC : NO_3 uptake and POC : PON build-up, as well as average cell numbers (mL^{-1}) and average TEP concentrations (μg GXeq L^{-1}) in the stationary phase with respective standard error (SE) of replicates ($n = 2$). Effects of temperature and CO_2 were assessed by PERMANOVA. Significant effects are in bold ($n = 2$, df = 1).

	Value (±SE)	Effect	SS	Pseudo-F	p (perm)
ΔDIC_{max}					
Ambient	333.2±3.2				
High Temp.	448.1±9.0	Temp.	14.8202	287.725	**0.004**
High CO_2	360.2±0.8	CO_2	0.5687	11.041	**0.036**
Combined	462.7±9.6	Temp.×CO_2	0.0740	1.438	0.360
ΔPOC_{max}					
Ambient	288.7±8.3				
High Temp.	412.0±14.0	Temp.	18.1083	112.407	**0.008**
High CO_2	314.7±13.1	CO_2	0.4054	2.517	0.179
Combined	418.1±3.5	Temp.×CO_2	0.1755	1.089	0.425
ΔDOC_{max}					
Ambient	11.6±2.4				
High Temp.	19.3±1.0	Temp.	1.1596	12.605	**0.013**
High CO_2	14.5±1.4	CO_2	0.0572	0.621	0.485
Combined	18.7±0.7	Temp.×CO_2	0.1140	1.239	0.345
$[\Delta DIC : \Delta NO_3]_{max}$					
Ambient	22.2±0.2				
High Temp.	29.9±0.6	Temp.	0.9880	287.631	**0.005**
High CO_2	24.0±0.1	CO_2	0.0379	11.036	**0.034**
Combined	30.8±0.6	Temp.×CO_2	0.0049	1.435	0.354
$[POC : PON]_{max}$					
Ambient	22.6±0.1				
High Temp.	30.6±0.5	Temp.	0.8699	274.266	**0.007**
High CO_2	22.5±0.4	CO_2	0.0335	10.559	**0.037**
Combined	27.9±0.5	Temp.×CO_2	0.0290	9.131	**0.039**
$Cell\ number_{avg\text{-}stat}$					
Ambient	33491±1646				
High Temp.	39717±1985	Temp.	469.3316	10.072	**0.042**
High CO_2	37218±2112	CO_2	159.5232	3.423	0.131
Combined	42950±3968	Temp.×CO_2	1.8382	0.039	0.818
$TEP_{avg\text{-}stat}$					
Ambient	2898.7±84.2				
High Temp.	4133.6±49.3	Temp.	199.6363	116.221	**0.003**
High CO_2	3333.2±183.8	CO_2	23.2679	13.546	**0.031**
Combined	4520.7±50.8	Temp.×CO_2	0.4369	0.254	0.607

(warming) μg GXeq L^{-1}, and 10,760 ± 1380 (combined) μg GXeq L^{-1} in the stationary phase. Thus, average TEP concentrations in the stationary phase were significantly decreased at higher temperatures, but elevated in the high CO_2 treatments (Fig. 3F; Table 2).

Stoichiometry

While the same amount of NO_3^- was consumed in all treatments and in both experiments, the uptake of DIC differed significantly across treatments and between experiments. In the *T. weissflogii* experiment, the uptake ratio of DIC over NO_3^- was significantly elevated in the treatments at high temperature and elevated pCO_2. In contrast, the consumption of carbon relative to nitrogen by *D. fragilissimus* decreased at higher temperatures, but was enhanced in the high CO_2 treatments (Tables 1, 2).

This elevated consumption of carbon over nitrogen is also reflected in the elemental ratios of produced organic matter (Fig. 4). The molar ratio of carbon to nitrogen (C : N) of POM under N-replete conditions remained between 8.0 and

Table 2. Effects of temperature and CO_2 on biogeochemical parameters in the experiment with *D. fragilissimus*. Shown are maximum net uptake of DIC, maximum net build-up of POC, and DOC (μmol C L^{-1}), maximum ratios of DIC : NO_3 uptake and POC : PON build-up, as well as average cell numbers (mL^{-1}) and average TEP concentrations (μg GXeq L^{-1}) in the stationary phase with respective standard error (SE) of replicates ($n = 2$). Effects of temperature and CO_2 were assessed by PERMANOVA. Significant effects are in bold ($n = 2$, df = 1).

	Value (±SE)	Effect	SS	Pseudo-F	p(perm)
ΔDIC_{max}					
Ambient	420.7±8.4				
High Temp.	271.6±11.6	Temp.	28.0617	204.642	**0.003**
High CO_2	516.9±10.7	CO_2	9.4978	69.264	**0.006**
Combined	358.4±9.1	Temp.×CO_2	0.0012	0.009	0.869
ΔPOC_{max}					
Ambient	298.3±19.5				
High Temp.	248.7±10.9	Temp.	2.2055	9.895	**0.045**
High CO_2	402.5±2.1	CO_2	14.1741	63.593	**0.005**
Combined	346.0±4.3	Temp.×CO_2	0.0958	0.429	0.538
ΔDOC_{max}					
Ambient	123.9±6.2				
High Temp.	70.0±8.3	Temp.	16.0904	22.938	**0.016**
High CO_2	136.4±24.5	CO_2	0.2271	0.324	0.595
Combined	63.2±0.3	Temp.×CO_2	1.4160	2.019	0.215
$[\Delta DIC : \Delta NO_3]_{max}$					
Ambient	28.0±0.6				
High Temp.	18.1±0.8	Temp.	1.8708	204.680	**0.003**
High CO_2	34.5±0.9	CO_2	0.6331	69.270	**0.006**
Combined	23.9±0.6	Temp.×CO_2	$8.30e^{-5}$	0.009	0.875
$[POC : PON]_{max}$					
Ambient	19.4±0.9				
High Temp.	16.5±0.7	Temp.	0.2545	17.128	**0.018**
High CO_2	24.0±0.9	CO_2	0.4724	31.790	**0.004**
Combined	20.6±0.6	Temp.×CO_2	$6.16e^{-5}$	0.004	0.908
$Cell\ number_{avg\text{-}stat}$					
Ambient	13009±40				
High Temp.	10149±387	Temp.	275.8384	24.846	**0.016**
High CO_2	16477±137	CO_2	505.2507	45.510	**0.005**
Combined	13991±1009	Temp.×CO_2	5.0633	0.456	0.503
$TEP_{avg\text{-}stat}$					
Ambient	11795.8±1525.3				
High Temp.	7944.4±513.9	Temp.	833.7093	13.860	**0.029**
High CO_2	15658.0±1062.2	CO_2	484.1105	8.048	**0.049**
Combined	10761.0±1379.1	Temp.×CO_2	2.5173	0.042	0.853

9.0 in both experiments, and was thus close to the Redfield value of 6.6. With the depletion of inorganic nitrogen, POC : PON increased rapidly in both experiments. Maximum values of POC : PON in the *T. weissflogii* experiment were significantly elevated at higher temperatures, whereas the effect of CO_2 was small and inconsistent at 15°C (Fig. 4A; Table 1). In contrast, POC : PON values in the *D. fragilissimus* experiment display a decrease at higher temperatures, but an increase at elevated pCO_2 (Fig. 4B; Table 2).

The C : N ratio of DOM was ~ 5.0 and 8.0 in the beginning of the experiment with *T. weissflogii* and *D. fragilissimus*, respectively. After N-depletion, DOC : DON slightly increased in the *T. weissflogii* experiment, however, without a detectable effect of temperature or pCO_2 (Fig. 4C). In the experiment with *D. fragilissimus*, DOC : DON was significantly elevated at lower temperatures, whereas an effect of pCO_2 could not be observed at either temperature (Fig. 4D).

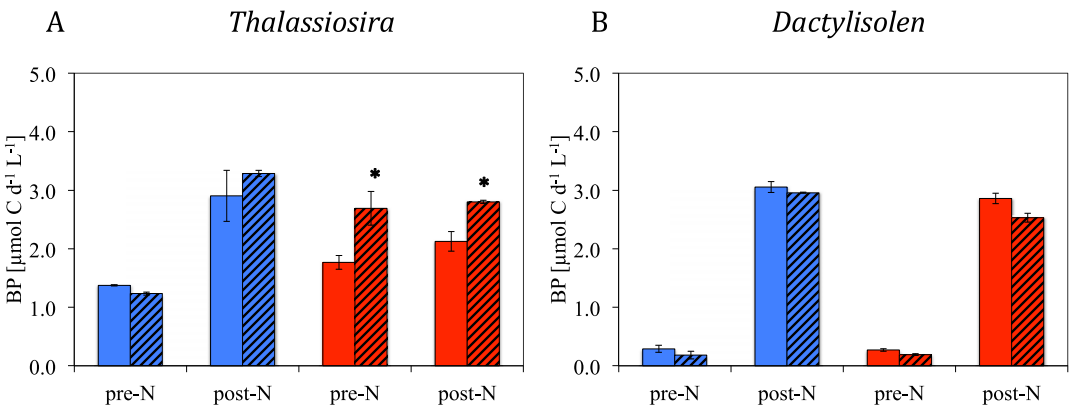

Fig. 5. Average rates of net BP in the period before and after nitrogen depletion (denoted "pre-N" and "post-N," respectively) in the experiment with (A) *T. weissflogii* and (B) *D. fragilissimus*) at 15 (blue) and 20°C (red), and in the ambient (solid) and high CO_2 treatment (hatched). Vertical error bars denote range of replicates within each treatment ($n = 2$). Asterisks denote significant effect of CO_2 in the respective temperature treatment ($p < 0.05$).

Bacterial activity

In the experiment with *T. weissflogii*, net BP in the 15°C treatments amounted to 1.30 ± 0.4 μmol C L^{-1} under N-replete conditions and increased to 3.09 ± 0.21 μmol C L^{-1} d^{-1} under N-depletion, irrespective of CO_2. In the 20°C treatments, however, BP was elevated under high CO_2 conditions displaying values of 1.77 ± 0.12 μmol C L^{-1} d^{-1} (ambient CO_2) and 2.69 ± 0.29 μmol C L^{-1} d^{-1} (high CO_2) during N-repletion and 2.13 ± 0.17 μmol C L^{-1} d^{-1} (ambient CO_2) and 2.80 ± 0.03 μmol C L^{-1} d^{-1} (high CO_2) in the period after N-depletion. A significant effect of temperature or CO_2 could not be detected when PERMANOVA was applied to both temperatures. However, CO_2 significantly enhanced BP in the *T. weissflogii* cultures at 20°C ($p < 0.05$) (Fig. 5A).

In the experiment with *D. fragilissimus*, BP increased from initial levels of 0.23 ± 0.02 μmol C L^{-1} d^{-1} under N-replete conditions to 2.85 ± 0.08 μmol C L^{-1} d^{-1} in the period after N-depletion, showing no significant difference between temperature and CO_2 treatments (Fig. 5B).

Discussion

Our results demonstrate that carbon uptake of the diatoms *T. weissflogii* and *D. fragilissimus* is markedly sensitive to CO_2 and temperature, and that this sensitivity is mainly driven by excess carbon fixation in the stationary phase under N-depletion. Carbon overconsumption has been observed in previous experiments and field studies (Banse 1994; Körtzinger et al. 2001; Sambrotto et al. 1993) and is usually associated with nutrient stress (Biddanda and Benner 1997; Wetz and Wheeler 2003). This is consistent with our experiments, where a substantial portion of inorganic carbon uptake and build-up of organic matter (up to 70%) by the diatom cultures occurred in the stationary phase: DIC uptake continued for several days after N-depletion and was even higher than during the period when inorganic N was available (in absolute terms), suggesting a strong decoupling of carbon and nitrogen cycling (Fig. 2C,D). Even though diatoms are known for luxury consumption of nutrients, i.e., storage inside their vacuole and delayed cell division and biomass production (Goldman et al. 1979), the magnitude of the stoichiometric decoupling observed in our experiments is remarkable. Interestingly, the sensitivity to CO_2 and temperature was most prominent in this period of N-limitation. Possible mechanisms behind that will be discussed below.

Note that *T. weissflogii* probably experienced colimitation by N and Si later in the experiment, whereas *D. fragilissimus* remained only N-limited throughout the experiment (Figs. 1-3). As Si is essential for cell division, this might have affected the biomass yield of *T. weissflogii* in the stationary phase and obscured possible effects of temperature and CO_2 on carbon uptake and partitioning in this experiment.

CO_2 effect on carbon uptake

Cumulative uptake of DIC by both diatom species was enhanced in the high CO_2 treatments, with the effect being much stronger on *D. fragilissimus* (+39%) than on *T. weissflogii* (+8%). This observation is in line with an earlier mesocosm experiment where DIC drawdown of a natural diatom-dominated community showed a marked increase under elevated pCO_2 (Riebesell et al. 2007). Remarkably, the fertilizing effect of CO_2 in our experiments was not detectable before N-depletion and when pCO_2 was still close to target levels of 400 μatm and 1000 μatm. Instead, most of the differences in DIC uptake occurred after N-depletion and when dissolved CO_2 dropped below $\sim$ 10 μmol L^{-1}, suggesting that inhibition of carbon assimilation at low concentrations of dissolved CO_2 played an important role in the observed response (Fig. 2C–F). This is in line with previous studies, which demonstrated that carbon acquisition in diatoms is highly sensitive to CO_2, especially at low concentrations of

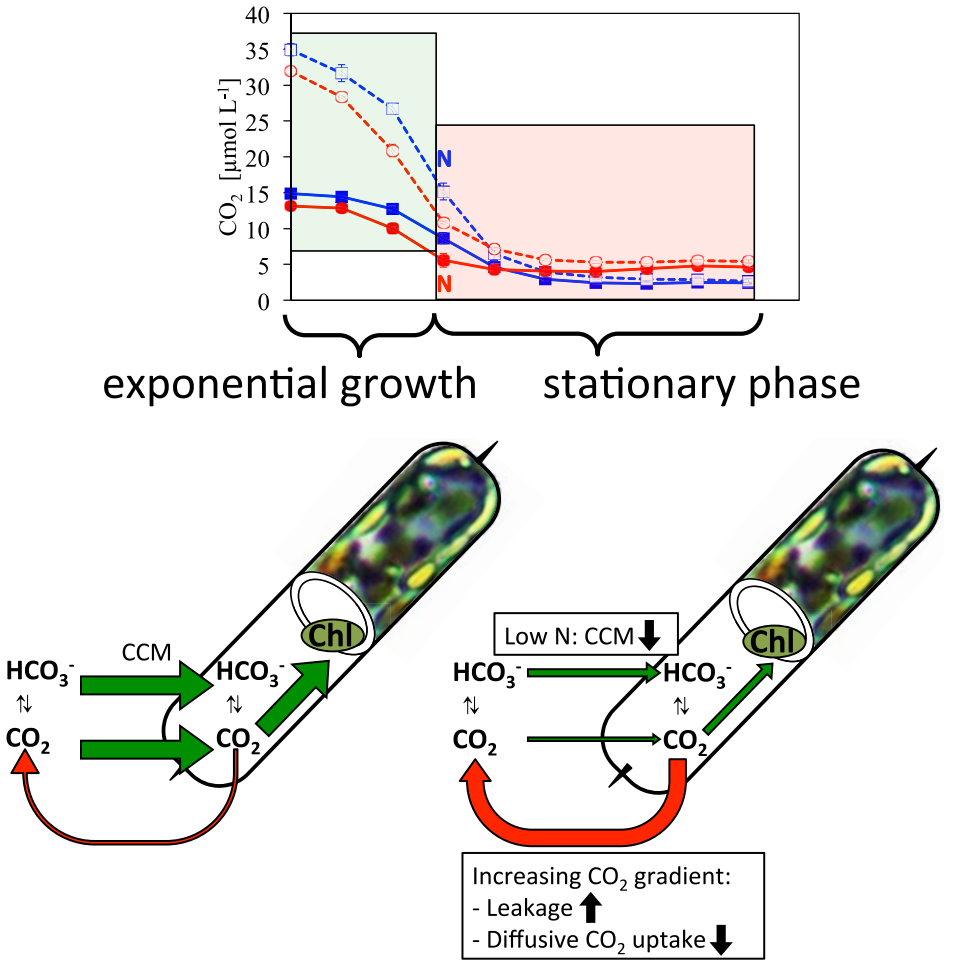

Fig. 6. Schematic diagram illustrating the influence of seawater CO_2 on carbon uptake by the diatoms over the course of the experiments. In the beginning of the experiment, CO_2 concentrations are relatively high and nutrients are available, leading to exponential growth of the diatoms (green square). Inorganic carbon is taken up either passively via diffusion of CO_2 or actively through a CCM, which transports HCO_3^- into the cell where it is subsequently converted to CO_2 and used for photosynthesis inside the chloroplast. Over the course of the experiment, CO_2 concentrations become very low and nitrogen gets depleted. In this stationary phase with low CO_2 and N-depletion, the CO_2 gradient between the cell interior and the surrounding seawater becomes so large that diffusive CO_2 uptake ceases and diffusive leakage of CO_2 becomes dominant. At the same time, active carbon uptake through the CCM decreases sharply, as there is a lack in nitrogen for the synthesis of CCM-specific proteins. Altogether, this leads to a situation in which the cells are no longer able to achieve a net uptake of inorganic carbon. Higher initial CO_2 conditions allowed the diatoms to take up more DIC until they reached such low CO_2 concentrations where carbon limitation occurred.

dissolved CO_2 (Riebesell et al. 1993; Burkhardt et al. 1999; Rost et al. 2003). Other studies also reported a close correlation of growth rate and POC production with dissolved CO_2, suggesting growth inhibition below 5–7.5 μmol CO_2 (Engel 2002; Bach et al. 2013).

Possible mechanisms behind the CO_2 effect

In our experiments, two mechanisms might have contributed to inhibition of carbon uptake at low CO_2: diffusive leakage of CO_2 from the cell and N-deficiency affecting the performance of the CCM.

Most phytoplankton species can actively take up HCO_3^- in addition to passive uptake of CO_2 via diffusion through operation of a CCM. Thereby, they enhance their intracellular CO_2 and HCO_3^-, which creates a concentration gradient between inside and outside the cell. However, photosynthetic carbon fixation by RubisCO is restricted to CO_2, which is why accumulated HCO_3^- has to be converted to CO_2 in the cell interior (Rost et al. 2003; Hopkinson et al. 2011). Thus, at decreasing concentrations of dissolved CO_2, the diffusion gradient between the cell interior and surrounding seawater increases strongly and CO_2 leakage becomes more and more dominant (Rost et al. 2006; Hopkinson et al. 2011; Bach et al. 2013).

At the same time, inhibition of carbon uptake at low CO_2 was likely also linked to N-limitation. As diffusive uptake of carbon becomes less efficient at lower CO_2 in seawater,

active carbon acquisition through a CCM gains importance. Diatoms possess a highly regulated and efficient CCM compared with some other phytoplankton groups (Burkhardt et al. 2001; Rost et al. 2003; Reinfelder 2011). However, the functioning of the CCM is ultimately controlled by the availability of nitrogen, which is required for the synthesis of specific proteins used in the CCM and can therefore be severely compromised by N-limitation (Geider et al. 1993; Giordano et al. 2005). With respect to our experiments, this implies that carbon limitation of the diatoms in the stationary phase was intensified by N-deficiency. As diatoms rely heavily on an efficient CCM for carbon acquisition, this effect became particularly pronounced at low CO_2 concentrations in seawater.

Altogether, these considerations indicate that diatoms in the high pCO_2 treatments could take up more DIC until they reached such low CO_2 concentrations, where carbon limitation occurred through the balance between uptake and leakage of inorganic carbon, and the exacerbating effect of N-limitation on the CCM (Fig. 6).

Interestingly, cell abundances were elevated in the high CO_2 treatments, with the effect being much more pronounced on *D. fragilissimus* (+40% cell concentrations) than on *T. weissflogii* (+10%) (Fig. 2A,B). This indicates that the observed CO_2 effect on DIC uptake stems from an increase in the carrying capacity of the system under high CO_2 rather than from a physiological response at the cellular level. Indeed, our data suggest that DIC uptake on a per cell basis was not considerably affected by CO_2 (calculated data not shown, see Fig. 2). Possibly, high CO_2 conditions resulted in a "memory effect" on the diatom cultures: higher CO_2 concentrations could have facilitated carbon acquisition to an extent that cells invested less resources in a CCM during the first few days of the experiment, and in turn had more resources left (for growth and cell division) in the phase of N-depletion and low CO_2 when carbon acquisition was complicated by the mechanism explained above. Thus, such a "memory effect" could explain the observation that although the differences in the carbonate systems of both treatments were largest during the first few days of the experiments, treatment effects became prominent much later when the differences between CO_2 in both treatments were small.

Differences among the species

The response to high CO_2 differed greatly between *T. weissflogii* and *D. fragilissimus*. While it cannot be excluded that this is partly due to secondary Si-limitation of *T. weissflogii*, it is likely that these CO_2-associated differences are either species-specific or related to cell size in general.

Species-specific differences seem obvious as photosynthetic pathways of diatoms are diverse and there are substantial differences regarding the preference for the form of inorganic carbon (CO_2 or HCO_3^-) among taxa (Rost et al. 2003; Giordano et al. 2005; Roberts et al. 2007). Considering that under ocean acidification conditions changes in dissolved CO_2 are much larger than those in HCO_3^-, it is likely that species more dependent on CO_2 as a carbon source, are more affected by changing pCO_2. Such species-specific variations could explain why *D. fragilissimus* displayed a much stronger reaction to high pCO_2 than *T. weissflogii*. The fact that the latter species did not show a strong sensitivity to CO_2 might also be linked to a C_4 pathway in their CCM. Previous work has demonstrated that *T. weissflogii* (and possibly other species) apply a biochemical CCM where HCO_3^- is transported into the cell and converted into an organic C4 compound by the C_4-carboxylase PEPCase prior to fixation by RubisCO (Reinfelder et al. 2000; Roberts et al. 2007).

Besides species-specific differences, the diverging response of the two species examined here could be related to cell size of the diatoms. In our study, the effect of CO_2 was much more prominent for the large, chain-forming *D. fragilissimus*, than for the rather small, unicellular *T. weissflogii*. Possibly, *D. fragilissimus* experienced a much stronger carbon limitation at low concentrations of dissolved CO_2 (as explained above) than *T. weissflogii*. Based on theoretical considerations, efficiency of carbon uptake should be related to cell size: small cells have a larger surface area per unit volume than large cells and can thus can support more transporters on the cell surface relative to their volume and maintain a thinner diffusion boundary layer (Wolf-Gladrow and Riebesell 1997; Finkel et al. 2010). In addition, larger cells with lower surface-to-volume ratios have to rely more heavily on a CCM than on diffusive uptake of CO_2 and could consequently be affected more severely by N-limitation of their CCM. These considerations are supported by recent findings from laboratory experiments, which revealed a pronounced size dependence of the growth-stimulating effect of CO_2 on diatoms (Wu et al. 2014). Similar observations have been made on natural, mixed phytoplankton assemblages, e.g., in the Ross Sea, where elevated CO_2 favored large chain-forming diatoms over small single-celled diatoms, resulting in an overall increase in carbon fixation of the phytoplankton community (Tortell et al. 2008).

As mentioned earlier, *T. weissflogii* reached Si-limitation in the stationary phase (Figs. 2, 3). This shortage in Si supply might have prevented cells from continued division, thereby possibly weakening bulk carbon uptake of the cultures and obscuring effects of CO_2 and temperature to a certain degree.

Temperature effect on carbon uptake

Temperature had an appreciable effect on the magnitude of DIC uptake and build-up of biomass by both diatom species (Figs. 2, 3). Warming from 15°C to 20°C resulted in a 31% increase in carbon uptake by *T. weissflogii*, but a 33% decrease by *D. fragilissimus*. This response is consistent with the results from the temperature-gradient experiments, which suggest different physiological optimum temperatures for the two species (Fig. 1). Consequently, the temperature rise from 15°C to 20°C in the main experiment had a

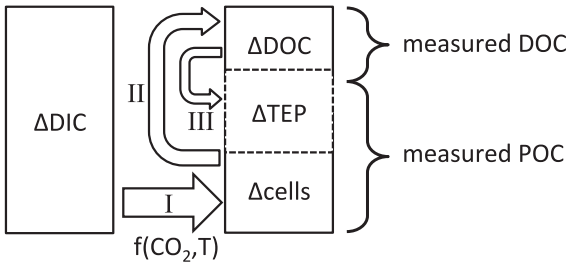

Fig. 7. Schematic diagram showing the influence of partitioning of DIC uptake into the different organic carbon pools. Diatom cultures fix inorganic carbon, which is modulated by CO_2 and temperature (I). With decreasing nutrient availability, an increasing portion of the fixed carbon is exuded as DOC (II). A certain portion of this DOC constitutes precursor material for TEP, which form abiotically (III) and can constitute a major fraction of POC. Measured standing stocks of DOC and POC, however, do not allow for a quantitative discrimination between cellular carbon and TEP or a determination of "true" DOC release that includes dissolved TEP precursor material. Thus, while effects of CO_2 and temperature on net carbon uptake can be easily detected in the data, possible effects on the relative partitioning between the pools are obscured through the various conversion processes between the pools, as well as bacterial degradation (not shown).

stimulating effect on *T. weissflogii*, but acted as a stressor on *D. fragilissimus* and pushed it beyond its temperature optimum.

It is well-known that temperature exerts a substantial influence on most cellular processes, such as enzymatic reactions. Consequently, growth and metabolic rates are enhanced by increasing temperatures until a species' optimum is reached. A further increase in temperature beyond this optimum is detrimental, leading to reduced growth rates, cellular damage, and eventually cell death (Raven and Geider 1988; Boyd et al. 2010; Finkel et al. 2010). While the effects of temperature on phytoplankton growth rates have been investigated in a number of previous studies (Eppley 1972; Boyd et al. 2013), implications for biogeochemical cycling have received only little attention. Our results show that the direction and magnitude of the effect, e.g., on net carbon uptake strongly depends on the temperature change relative to the temperature range and optimum of the investigated species. In fact, such species-specific differences in temperature sensitivity were identified as the main driver behind seemingly contradictory effects of warming on carbon cycling by natural plankton communities in earlier mesocosm studies (Wohlers et al. 2009; Taucher et al. 2012). These studies demonstrated that the physiological response of the dominant species can determine the temperature effect on the entire plankton community. Another factor that could have possibly affected carbon uptake to in our experiments is the effect of temperature on the buffer capacity of seawater (Sundquist et al. 1979). At 20°C, the same amount of DIC uptake is accompanied by smaller changes in pCO_2 and pH than at 15°C. Thus, an elevated buffer capacity at higher temperatures might increase the amount of DIC that can be taken up by phytoplankton until CO_2 limitation is reached. However, as the temperature response differed severely between the two diatom species in this study, it is likely that the direct physiological effect of temperature was dominant and overshadowed the possible effects of temperature-driven changes in the buffer capacity.

Fate of carbon overconsumption: Partitioning of organic matter and the role of TEP

DIC uptake was accompanied by a substantial build-up of organic carbon in both experiments that largely mirrored the respective effects of temperature and CO_2 on the two diatom species (Fig. 3). Our data indicate that the partitioning of fixed carbon between the particulate and dissolved pool as well as the effects of CO_2 and temperature, are tightly linked to the formation of gel-like TEP.

It is well known that carbon overconsumption after nutrient depletion is mainly released as DOC, thereby acting as a "carbon overflow" mechanism that helps to preserve the metabolic functionality of the cell when there is an imbalance between light and nutrients (Fogg 1983). Previous studies have shown that abiotic formation can transform a substantial fraction of DOC exudates into TEP on very short timescales (Passow 2000; Engel et al. 2004). With respect to our experiments, this suggests that a large portion of organic matter that was originally released as DOC comprised TEP-precursors, which were then rapidly transformed into TEP (Fig. 3E,F). This is consistent with previous studies, which demonstrated that TEP can constitute a substantial fraction of total POC (Engel et al. 2004; Wetz and Wheeler 2007). It also illustrates how TEP formation affects the interpretation of organic matter partitioning by underestimating true DOC production and overestimating the amount of organic matter retained as cellular carbon. Due to these mechanisms it cannot be resolved whether the effects of temperature and CO_2 on DIC uptake simply translated into the build-up of the different organic carbon pools, or if there were additional direct effects on the relative partitioning between cellular carbon, DOC and TEP (Fig. 7).

Organic carbon partitioning between the particulate and dissolved phase differed notably among species in the experiments presented here. While inorganic carbon uptake by *T. weissflogii* could almost entirely be traced to the POC pool (~ 95%), a much larger fraction of DIC uptake accumulated as DOC (20–45%) in the experiment with *D. fragilissimus* (Fig. 3). These differences might be attributable to variations in the amount and quality of produced DOC, the fraction of TEP-precursors contained in this DOC, and their subsequent conversion to TEP (Fig. 7). Such differences might also explain why some studies found carbon overconsumption to be channeled mainly into an increase in DOC (Sambrotto et al. 1993; Koeve, 2004), whereas others could trace this

additional carbon almost entirely to POC and TEP (Engel et al. 2002; Wetz and Wheeler 2007).

The substantial formation of TEP, which are very carbon-rich and usually have C : N ratios of >20 (Mari et al. 2001), can explain most of the high POC : PON ratios of up to 25 found in our experiments (Fig. 4). However, changes in the cellular composition might have additionally contributed to the observed increase in POC : PON. Particularly in the experiment with *D. fragilissimus*, maximum cell concentrations varied strongly with CO_2 and temperature, even though the initial amount of inorganic nitrogen was identical. These differences in cell numbers must be linked with changes in their elemental composition, which can be highly variable in diatoms especially under nutrient starvation (Brzezinski 1985; Goldman et al. 1992; Harrison et al. 1977). Such variations can be driven by production of cells with decreasing nitrogen content under N-limitation or an increase in the intracellular content of carbon-enriched compounds, such as lipids and carbohydrates (Goldman et al. 1992; McGinnis et al. 1997; Bach et al. 2013).

Furthermore, it has to be noted that the temporal development of measured DIC, POC, and DOC must be considered as net change. It is likely that actual DOC release was underestimated due to bacterial respiration. In our experiments, we resolved net BP. Even though measured BP did not exceed 3 μmol L^{-1} d^{-1}, bacterial carbon demand (i.e., the gross flux of carbon through the bacteria pool) could have been higher, depending on the bacterial growth efficiency (BGE). Because DOC build-up by *T. weissflogii* was relatively low ($<$ 20 μmol L^{-1}, Table 1), slight differences in bacterial respiration might have a notable effect on measured DOC accumulation. BP was elevated in the high CO_2 treatments of *T. weissflogii* (Fig. 5A), which is in line with previous studies that found a positive effect of elevated CO_2 on bacterial activity (Grossart et al. 2006; Endres et al. 2014). While we do not have information about BGE, elevated bacterial carbon demand could have obscured the visible effect of high CO_2 on accumulation of DOC in this experiment. In contrast, DOC build-up by *D. fragilissimus* was much higher ($\sim$ 60-130 μmol L^{-1}, Table 2) and net BP was similar across all treatments (Fig. 5B). Thus, while heterotrophic bacterial consumption could have led to an underestimate of true DOC production, it is not clear, without more information about BGE, if bacterial carbon demand obfuscated a CO_2 effect on DOC exudation in the *D. fragilissimus* experiment. It should also be noted that observed differences in bacterial activity among treatments might be largely driven by indirect effects, such as differences in the quality of produced substrate by the diatoms (e.g., DOC), than by direct effects of temperature and CO_2 on metabolic rates of bacteria.

Altogether, both the formation of TEP from dissolved precursor material, as well as uncertainties about bacterial carbon demand could have led to an underestimation of true DOC release and obscured the effects of temperature and CO_2 on carbon partitioning in our experiments (Fig. 7).

Potential implications for the marine carbon cycle

Our experiments revealed a notable sensitivity of carbon uptake and production of organic matter of two diatom species to both temperature and CO_2, particularly in the stationary phase after nitrogen was depleted. Remarkably, up to 70% of DIC uptake occurred after N-depletion. Although previous studies concluded that CO_2 is usually not a limiting factor for photosynthetic carbon fixation, with only negligible impacts of elevated CO_2 on diatoms, our results demonstrate that the effect becomes much more prominent when considering temporal bloom dynamics and especially the stationary phase. We show that a higher initial pCO_2 can substantially increase the amount of inorganic carbon that is taken up over the course of a diatom bloom before CO_2 becomes a limiting factor: net DIC uptake in our experiments was enhanced by 8–39% under high CO_2 conditions. At the same time, our study illustrates that the same magnitude of warming can have either stimulating or inhibiting effects on carbon uptake and production of biomass by diatoms that co-occur in nature, depending on their species-specific temperature range.

The magnitude of these observed responses is quite significant, considering that changes in CO_2 and temperature as in our experiment can be expected by the end of this century. Thus, the observed effects on the diatoms could act as a feedback mechanism in the marine carbon cycle. Whether the sensitivity of carbon uptake to temperature and CO_2 presented in this study results in a negative or positive climate feedback would depend on the fate of the organic carbon. It has been shown that DOC can significantly contribute to the carbon flux to the deep ocean through transport via deep water formation and mixing (Hansell and Carlson 1998; Carlson et al. 2010). An increase in DOC release by phytoplankton could therefore enhance the transport of carbon from the atmosphere to the ocean interior, depending on the quality and bioavailability of the produced DOC (Carlson and Hansell 2014). If the additional DOC gets rapidly remineralized in the surface ocean, the net effect on carbon flux would be zero. Thus, more information on degradability of DOC release by phytoplankton is required. Even stronger effects on the biological pump could be mediated by changes in the production of TEP, which facilitate the formation of marine aggregates through their high stickiness and thus play an important role in particle flux to the ocean interior (Alldredge et al. 1993; Logan et al. 1995; Passow 2002). Our results suggest a substantial sensitivity of TEP formation to temperature and CO_2 : TEP accumulation in the stationary phase was profoundly enhanced under high CO_2 conditions (+12% for *T. weissflogii* and +35% for *D. fragilissimus*) and also displayed a substantial response to warming from 15°C to 20°C (+40% *T. weissflogii* and −30% by *D. fragilissimus*). Such a significant increase in

TEP production could promote the formation of fast-sinking aggregates, thereby enhancing the particle flux out of the euphotic zone, and ultimately the biological pump (Arrigo 2007).

However, sensitivities to temperature and CO_2 seem to be highly variable among species, making it difficult to predict whether the net effect of ocean warming and acidification will be an enhancement of the biological pump or an acceleration of the microbial loop. The outcome for entire pelagic ecosystems will depend on the sensitivities of the different ecosystem components and its most important species, as these effects will ultimately propagate into community composition, food web dynamics, and the efficiency of the biological pump. As the major fraction of DIC uptake and organic matter build-up in our experiments occurred after N-depletion, we suggest that more attention should be paid to the stationary phase and post-bloom dynamics in future studies, especially on the ecosystem level (e.g., mesocosm studies).

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