Iron Limitation Modulates Ocean Acidification Effects on Southern Ocean Phytoplankton Communities

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Abstract

The potential interactive effects of iron (Fe) limitation and Ocean Acidification in the Southern Ocean (SO) are largely unknown. Here we present results of a long-term incubation experiment investigating the combined effects of CO₂ and Fe availability on natural phytoplankton assemblages from the Weddell Sea, Antarctica. Active Chl a fluorescence measurements revealed that we successfully cultured phytoplankton under both Fe-depleted and Fe-enriched conditions. Fe treatments had significant effects on photosynthetic efficiency (Fm'/Fm), 0.3 for Fe-depleted and 0.5 for Fe-enriched conditions), non-photochemical quenching (NPQ), and relative electron transport rates (rETR). pCO₂ treatments significantly affected NPQ and rETR, but had no effect on Fm'/Fm. Under Fe limitation, increased pCO₂ had no influence on C fixation whereas under Fe enrichment, primary production increased with increasing pCO₂ levels. These CO₂-dependent changes in productivity under Fe-enriched conditions were accompanied by a pronounced taxonomic shift from weakly to heavily silicified diatoms (i.e. from Pseudo-nitzschia sp. to Fragilariopsis sp.). Under Fe-depleted conditions, this functional shift was absent and thinly silicified species dominated all pCO₂ treatments (Pseudo-nitzschia sp. and Synedropsis sp. for low and high pCO₂ respectively). Our results suggest that Ocean Acidification could increase primary productivity and the abundance of heavily silicified, fast sinking diatoms in Fe-enriched areas, both potentially leading to a stimulation of the biological pump. Over much of the SO, however, Fe limitation could restrict this possible CO₂ fertilization effect.

Introduction

The Southern Ocean (SO) exerts a disproportionate control on the global carbon cycle over glacial-interglacial timescales [1,2] and contributes significantly to the oceanic sequestration of anthropogenic CO₂ [3]. Besides abiotic drivers such as ocean circulation and sea-ice cover, biological carbon uptake and drawdown also control the air-sea-flux of CO₂ in the SO [1,2]. These biological processes are mediated by phytoplankton communities, dominated mainly by silicifying diatoms [4].

The surface waters of the SO are rich in major nutrients such as nitrate and phosphate, but in vast areas of this region primary production is limited by low iron (Fe) availability [5]. Both laboratory and in-situ fertilisation experiments have demonstrated that the growth of SO phytoplankton is strongly enhanced by the addition of Fe [6,7,8]. As Fe is a key nutrient for biochemical pathways including photosynthesis and nitrate assimilation [9], limiting Fe concentrations lead to decreased photochemical efficiencies and photosynthetic rates [10,11]. One important source of iron in open-ocean waters is the melting of sea-ice, which causes seasonal and localised phytoplankton blooms and strong vertical particle fluxes [12,13]. These factors make the marginal sea-ice zone a biogeochemically important region of the SO [12].

The effects of seawater carbonate chemistry on SO phytoplankton have received increasing attention over recent years. Laboratory studies suggest that Antarctic phytoplankton can be growth-limited by CO₂ supply under present-day CO₂ concentrations [14,15]. Field data from continental shelf waters of the Ross Sea have demonstrated CO₂-dependent changes in primary productivity and phytoplankton assemblages [16,17]. In these prior studies, phytoplankton assemblages were not demonstrably Fe-limited (e.g. high Fm'/Fm reported in [17]), making the extrapolation of results to the open SO waters difficult. Recently, pH-dependent shifts in Fe speciation have been reported [18], suggesting a strong potential for ocean acidification (OA) to reduce Fe bioavailability as seen in experiments with Arctic phytoplankton assemblages [19].

Given the Fe-limited status of much of the SO, there is a great need to investigate combined effects of OA and Fe limitation in this region. Here we present results from a CO₂-Fe-incubation experiment (190, 390 and 800 μatm pCO₂ under Fe-enriched and
Fe-depleted conditions) using an open ocean phytoplankton assemblage from the Weddell Sea, an important region for SO primary productivity. The aim of this study was to investigate the interactive effects of OA and Fe availability on species composition, primary production, as well as iron uptake and photosynthesis of Fe-limited phytoplankton assemblages.

**Materials and Methods**

**Experimental Setup**

A ship-board incubation experiment was designed using a CO₂-Fe-matrix approach to examine potential interactive effects between CO₂ and Fe availability on SO phytoplankton communities. A natural phytoplankton assemblage from the Weddell Sea (66°50′S, 0°W) was sampled during mid Dec. 2010 on the RV *Polarstern* ANT-XXVII/2 cruise. The permission for field work according to the Antarctic Treaty was issued by the Umweltbundesamt (Germany). Seawater was collected from 30 m depth using a “torpedo fish” towed outside the ship’s wake [20]. To eliminate large grazers, we filtered seawater through an acid-cleaned 200 µm mesh. Water containing the natural phytoplankton community was transferred into acid-cleaned 4L polycarbonate bottles and incubated in growth chambers at 3±1°C with a constant daylight irradiance of 40±5 µmol photons m⁻² s⁻¹ (Master TL-D 18W daylight lamps, Philips, adjusted by neutral density screens). The applied irradiance was based on several light measurements in the SO at the sampling depth (data by Mitchell; e.g. DOI: 10.1594/PANGAEA.132802). To provide sufficient time for changes in the phytoplankton assemblages to occur and to achieve ecologically relevant information, experiments lasted between 18 and 30 days depending on experimental treatment (18–20 days in case of Fe-enriched and 27–30 days in case of Fe-depleted treatments). In order to prevent significant changes in chemical conditions due to phytoplankton growth, incubations were diluted with 0.2 µm filtered seawater when nitrate concentrations were about 10 µmol kg⁻¹. Dilution water was obtained from the initial sampling location and filtered through acid-cleaned 0.2 µm filter cartridges (AcroPak 1500, Pall). Experiments were run with triplicate treatments of two Fe levels (Fe-enriched and Fe-depleted; see below) and 3 pCO₂ levels (190, 390 and 800 µatm).

Tubing, bubbling systems, reservoir carboys, incubation bottles and other equipment were acid-cleaned prior to the cruise using trace metal-clean techniques: After a 2-day Citranox detergent bath and subsequent rinsing steps with Milli-Q (MQ, Millipore), Tubing, bubbling systems, reservoir carboys, incubation bottles and other equipment were acid-cleaned prior to the cruise using trace metal-clean techniques: After a 2-day Citranox detergent bath and subsequent rinsing steps with Milli-Q (MQ, Millipore), equipment was kept in acid (5 N HCl for polyethylene and 1 N HCl for polycarbonate materials) for 7 days, followed by 7 rinses with MQ. Equipment was kept triple-bagged during storage and experiments. Incubation bottles were stored under acidified conditions (addition of 500 µL 10 N suprapure quartz distilled HCl; Carl Roth, in 500 mL MQ and rinsed twice with seawater prior to the start of the experiment.

In order to mimic different pCO₂ conditions, the incubation bottles were continuously sparged with air of different CO₂ partial pressures (190, 390 and 800 µatm) delivered through sterile 0.2 µm air-filters (Midisart 2000, Sartorius stedim). Gas mixtures were generated using a gas flow controller (CGM 2000 MCZ Umweltechnik), in which CO₂-free air (<1 ppmv CO₂; Dominick Hunter) was mixed with pure CO₂ (Air Liquide Deutschland). The CO₂ concentration in the mixed gas was regularly monitored with a non-dispersive infrared analyzer system (LI6252, LI-COR Biosciences) calibrated with CO₂-free air and purchased gas mixtures of 150±10 and 1000±20 ppmv CO₂ (Air Liquide Deutschland). To promote phytoplankton growth, 1 nM Fe (FeCl₃, ICP-MS standard, TraceCERT, Fluka) was added to the Fe-enriched treatments. In the Fe-depleted treatments, 10 nM of the hydroxamato siderophore desferrioxamine B (DFB, Sigma) was added to bind and thereby reduce the bioavailable Fe [21,22]. No additional macronutrients were added to the incubation bottles. Abiotic control bottles, used to assess changes in Fe chemistry, contained filtered seawater (0.2 µm) exposed to each treatment condition over the duration of the experiment.

**Chemical parameters**

Nutrients were determined colorimetrically on-board with a Technicon TRAACS 800 Auto-analyzer on a daily basis over the course of the experiments, following procedures improved after [23]. Samples for total alkalinity (TA) were 0.6 µm-filtered (glass fibre filters, GF/F, Whatman), fixed with 0.03% HgCl₂ and stored in 150 mL borosilicate bottles at 4°C. TA was estimated at the Alfred Wegener Institute (Germany) from duplicate potentiometric titration [24] using a TitroLine alpha plus (Schott Instruments). The calculated TA values were corrected for systematic errors based on measurements of certified reference materials (CRMs provided by Prof. A. Dickson, Scripps, USA; batch #111; reproducibility ±5 µmol kg⁻¹). Dissolved inorganic carbon (DIC) samples were filtered through 0.2 µm cellulose-acetate filters (Sartorius stedim), fixed with 0.03% HgCl₂ and stored in 5 mL gas-tight borosilicate bottles at 4°C. Also in the home laboratory, DIC was measured colourimetrically in triplicate with a QuAAtro autoanalyzer (Seal) [25]. The analyser was calibrated with NaHCO₃ solutions (with a salinity of 35, achieved by addition of NaCl) with concentrations ranging from 1800 to 2300 µmol DIC kg⁻¹. CRMs were used for corrections of errors in instrument performance (e.g. baseline drift). Seawater pH was measured potentiometrically on the NBS scale (pH₃/5 overall uncertainty 0.02 units) with a two-point calibrated glass reference electrode (IONline, Schott Instruments). Values for pH were reported on the pH_total scale for better comparability with other datasets. Following suggestions by Hoppe et al. [26], seawater carbonate chemistry (including pCO₂) was calculated based on TA and pH using CO₂SYS [27]. The dissociation constants of carbonic acid of Mehrbach et al. (refit by Dickson and Millero) were used for calculations [28,29]. Dissociation constants of carbonic acid of Mehrbach et al. (refit by Dickson and Millero) were used for calculations [28,29]. Dissociation constants of carbonic acid of Mehrbach et al. (refit by Dickson and Millero) were used for calculations [28,29]. Dissociation constants of carbonic acid of Mehrbach et al. (refit by Dickson and Millero) were used for calculations [28,29]. Dissociation constants of carbonic acid of Mehrbach et al. (refit by Dickson and Millero) were used for calculations [28,29]. Dissociation constants of carbonic acid of Mehrbach et al. (refit by Dickson and Millero) were used for calculations [28,29]. Dissociation constants of carbonic acid of Mehrbach et al. (refit by Dickson and Millero) were used for calculations [28,29]. Dissociation constants of carbonic acid of Mehrbach et al. (refit by Dickson and Millero) were used for calculations [28,29]. Dissociation constants of carbonic acid of Mehrbach et al. (refit by Dickson and Millero) were used for calculations [28,29]. Dissociation constants of carbonic acid of Mehrbach et al. (refit by Dickson and Millero) were used for calculations [28,29]. Dissociation constants of carbonic acid of Mehrbach et al. (refit by Dickson and Millero) were used for calculations [28,29]. Dissociation constants of carbonic acid of Mehrbach et al. (refit by Dickson and Millero) were used for calculations [28,29]. Dissociation constants of carbonic acid of Mehrbach et al. (refit by Dickson and Millero) were used for calculations [28,29]. Dissociation constants of carbonic acid of Mehrbach et al. (refit by Dickson and Millero) were used for calculations [28,29]. Dissociation constants of carbonic acid of Mehrbach et al. (refit by Dickson and Millero) were used for calculations [28,29]. Dissociation constants of carbonic acid of Mehrbach et al. (refit by Dickson and Millero) were used for calculations [28,29].
cells were collected onto precombusted GF/F-filters (15 h, 500°C), which were subsequently stored at −20°C and dried for >12 h at 60°C prior to sample analysis. Analysis was performed using an Automated Nitrogen Carbon Analyser mass spectrometer system (ANCA-SL, 20-20, SerCon Ltd.). Samples for determination of chlorophyll a (Chl a) concentration were filtered onto 0.45 μm cellulose acetate filters (Sartorius stedim) and stored at −20°C until analysis onboard. Chl a was subsequently extracted in 10 mL 90% acetone (overnight in darkness, at 4°C) and concentrations determined on a fluorometer (10-000 R, Turner Designs), using an acidification step to determine phaeopigments [35].

Physiological assays

Primary production of the final phytoplankton assemblages was determined in 100 mL incubations after addition of a 10 μCi (0.37 MBq) spike of NaH\(^{14}\)CO\(_3\) (PerkinElmer, 33.1 mCi mmol\(^{-1}\)). From the incubations, 0.5 mL aliquots were immediately removed and mixed with 10 mL of scintillation cocktail (Ultima Gold AB, PerkinElmer) to determine the total amount of added NaH\(^{14}\)CO\(_3\). For blank determination, sample filters were added at the end of the experiment (Table 1), indicating that the apparent maximum quantum yield of photochemistry in PSII (F\(_{\text{PSII}}\)) fluorescence of the MTF was used in order to fully reduce the PSII and the plastoquinone (PQ) pool. The minimum (F\(_{0}\)) of the STF phase and maximum (F\(_{m}\)) fluorescence of the MTF was used to calculate the apparent maximum quantum yield of photochemistry in PSII (F\(_{\text{PSII}}\)) according to the equation \(F_{m} - F_{0}/F_{m}\). This parameter was calculated for all bottles on a regular basis (every 6–7 days). Values of these parameters as well as of the functional absorption cross section of PSII (\(\Phi_{\text{PSII}} A^{2}\) quanta\(^{-1}\)) were derived using the Firere software provided by Satlantic [37]. Additional fluorescence measurements were performed under increasing irradiances (21, 41, 66, 88, 110 and 220 μmol photons m\(^{-2}\) s\(^{-1}\)) provided by an external actinic light source (warm white 350 mA LEDs, Illas3003, CML Innovative Technologies). After 5 minutes acclimation to the respective light level, the light-acclimated minimum (F\(_{\text{min}}\)) and maximum (F\(_{\text{max}}\)) fluorescence were estimated. The effective quantum yield of photochemistry in open reaction centers of PSII was derived according to the equation \(F_{\text{min}} - F_{\text{min}}'/F_{\text{max}}\cdot F_{\text{min}}'\) [38]. Relative electron transport rates (rETR) were then calculated as the product of effective quantum yield and applied growth irradiance of 40 μmol photons m\(^{-2}\) s\(^{-1}\). Using the Stern-Volmer equation [39], NPQ of Chl a fluorescence under growth irradiance was calculated as F\(_{\text{min}}'/F_{\text{max}} - 1\). NPQ was relaxed (values <0.1) at lowest light levels for all treatments (data not shown). All measurements were conducted at the growth temperature.

Statistics

All data is given as the mean of the replicates (n = 3) with 1 standard deviation. To test for significant differences between the treatments, Two Way Analyses of Variance (ANOVA) with additional normality tests (Shapiro-Wilk; passed for all data shown) were performed. The significance level was set to 0.05. Statistical analyses were performed with the program SigmaPlot (SysStat Software Inc).

Results

Seawater chemistry

The initial carbonate system (pH: 7.93±0.01; DIC 2210±17 μmol kg\(^{-1}\); TA: 2303±14 μmol kg\(^{-1}\)) shifted to experimental treatment levels (average pH of 8.39±0.02, 8.13±0.02, and 7.80±0.03 for the three CO\(_2\) treatments) within the first 2 days of the experiment (Figure 1A). The semi-continuous dilute-batch approach led to stable seawater carbonate chemistry over the course of the experiment (Figure 1A). Compared to abiotic controls, drift was <8% and <5% for TA and DIC, respectively (Table 1). Initial seawater nutrient concentrations were 29 μmol kg\(^{-1}\) nitrate, 76 μmol kg\(^{-1}\) silicate and 2 μmol kg\(^{-1}\) phosphate. Over the course of the experiment, concentrations of nitrate never fell below 7 μmol kg\(^{-1}\), while silicate and phosphate concentrations were always above 40 and 0.8 μmol kg\(^{-1}\), respectively. Initial Fe concentration in the water sampled for incubations was 1.12±0.15 nmol kg\(^{-1}\). In 0.2 μm filtered seawater (i.e. abiotic control treatments) enriched with 10 nM DFB, dissolved Fe concentrations remained 1.16±0.08 nmol kg\(^{-1}\) until the end of the experiment (Table 1), indicating that the experimental bottles remained free of Fe contamination. Dissolved Fe concentrations decreased in Fe-enriched seawater (1 nM Fe added, Table 1).

Photophysiology

Over the course of the experiment, we observed significant Fe-dependent differences in the apparent maximum quantum yield of PSII reaction centres (F\(_{\text{r}}\)/F\(_{\text{m}}\); Figure 1 B; p<0.001). Average values of F\(_{\text{r}}\)/F\(_{\text{m}}\) at the end of the experiment were 0.51±0.04 and
0.32±0.03 for Fe-enriched and -depleted treatments, respectively (Table 2). In contrast, the pCO₂ treatments had no effects on Fv/Fm under either of the Fe treatments. Relative electron transfer rates from PSII (rETR) were significantly higher in Fe-enriched treatments, and also showed pCO₂-dependent increases under both Fe-enriched (5.7±0.8 at low and 7.5±0.9 at high pCO₂; p<0.001) and Fe-depleted conditions (4.2±0.4 at low and 5.3±0.1 at high pCO₂; p<0.001). Significant interactive effects between Fe- and pCO₂-treatments were also observed in photoprotective non-photochemical quenching (NPQ; Table 2; p<0.001). Under Fe-limitation, NPQ decreased from 0.22±0.03 at low pCO₂ to 0.11±0.00 at high pCO₂, while under Fe-enriched conditions, NPQ was independent of pCO₂ (0.06±0.01 at all pCO₂ levels; p<0.001).

The ratios of Chl a to POC of the final phytoplankton assemblages (Table 1) were significantly higher (p<0.001) in Fe-enriched (0.023±0.003 at low and 0.017±0.004 at high pCO₂) compared to Fe-depleted treatments (0.012±0.001 at low and 0.010±0.001 at high pCO₂). Chl a/POC ratios furthermore decreased significantly with increasing pCO₂ levels (p = 0.012), irrespective of the Fe-status.

Fe and C uptake
For all pCO₂ levels, carbon-normalized Fe uptake capacities at the end of the experiment were 10-fold higher in Fe-depleted compared to Fe-enriched treatments (Figure 2; p<0.001), but with no significant CO₂ effect. Combining data across pCO₂ treatments, mean Fe uptake capacities were 7.50±3.35 pmol Fe (µmol POC⁻¹ h⁻¹) and 0.72±0.38 pmol Fe (µmol POC⁻¹ h⁻¹) at low and high Fe, respectively. Under Fe-enriched conditions, we observed a significant CO₂-dependent increase in C-specific primary productivity (Figure 3; p<0.001). Primary productivity increased from 4.21±0.44 nmol C (µmol POC⁻¹ h⁻¹) at low pCO₂ to 8.15±0.75 nmol C (µmol POC⁻¹ h⁻¹) at high pCO₂. In contrast, no CO₂-dependent productivity responses were observed in Fe-depleted treatments, with values of 3.62±0.38 nmol C (µmol POC⁻¹ h⁻¹) at low pCO₂ and 3.93±0.16 nmol C (µmol POC⁻¹ h⁻¹) at high pCO₂. Thus, there was a significant interactive effect of the CO₂- and Fe-treatments on NPP (p = 0.023).

Species composition
We observed pronounced shifts in the diatom-dominated phytoplankton assemblages in association with the CO₂-dependent changes in primary productivity (Figure 4; Table 3). Shifts in species composition did not lead to changes in average cell size in the different assemblages (data not shown). After Fe-enrichment, *Pseudo-nitzschia* cf. *turgiduloides* was the most abundant species under low and intermediate pCO₂ (39.2±5% and 40±9%, respectively), whereas *Pigulaeopsis cylindrus* dominated communities under high pCO₂ levels (72±5%). Furthermore, *Chuioceros cf. simplex* abun-

### Table 1. Seawater chemistry.

| Treatment       | Fe<sub>aq</sub> [µmol kg⁻¹] | DIC [µmol kg⁻¹] | TA [µmol kg⁻¹] | pH | pCO₂ [µatm] |
|-----------------|------------------------------|-----------------|---------------|----|-------------|
| Initial seawater| 1.12                         | ±0.15           | 2071          | 2271| 7.93        |
| +Fe 190 µatm CO₂| 0.45                         | ±0.07           | 2002          | ±11| 2208        |
|                | 390 µatm CO₂                 | 0.32            | ±0.04         | 2082| ±4          |
|                | 800 µatm CO₂                 | 0.21            | ±0.03         | 2175| ±14         |
| −Fe 190 µatm CO₂| 1.13                         | ±0.16           | 2018          | ±18| 2209        |
|                | 390 µatm CO₂                 | 1.25            | ±0.21         | 2096| ±41         |
|                | 800 µatm CO₂                 | 1.11            | ±0.09         | 2155| ±41         |

Parameters of the seawater carbonate chemistry were sampled at the beginning (n=1) and the end of the experiment (n=3; mean ± 1 s.d.). Total dissolved Fe measurements in abiotic control treatments after 0.2 µm filtration as measured by voltammetry (n = 2; mean ± 1 s.d.). The decreased dissolved Fe concentration in the +Fe treatment can be attributed to precipitation/absorption of colloidal iron. pCO₂ was calculated from TA and pH<sub>total</sub> at 3°C and a salinity of 34 using CO₂SYS [27], using average final nutrient concentrations of 1 and 60 µmol kg⁻¹ for phosphate and silicate, respectively.

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Figure 1. pH and Fv/Fm over the course of the experiment. Experimental conditions over the course of the experiment. A: Development of pH<sub>total</sub> (n = 3; mean ± 1 s.d.) in Fe-enriched (solid circles, 190 µatm CO₂; solid squares, 390 µatm CO₂; solid triangles, 800 µatm CO₂) and Fe-depleted treatments (open circles, 190 µatm CO₂; open squares, 390 µatm CO₂; open triangles, 800 µatm CO₂). B: Development of dark-adapted Fv/Fm (n = 3; mean ± 1 s.d.) in Fe-enriched (solid squares) and Fe-depleted treatments (open squares).
dances increased with rising CO₂ (from 11\% to 17\% at high pCO₂). Phytoplankton composition changes were observed significantly from those seen under high pFe conditions. Synedropsis sp., the most prevalent species under high Fe-depleted conditions, dominated the low pCO₂ treatment (35\% ± 10\%), while Syndrillaria sp. was the most prevalent species under high pFe conditions. Syracosphaera sp. was the most prevalent species under high pCO₂ conditions.

Table 2. Physiological differences between treatments.

| Treatment | Chl a: POC | F_v/F_m | NPQ | rETR |
|-----------|-----------|---------|-----|------|
| +Fe       | 190 μatm CO₂ | 0.023 ± 0.003 | 0.55 ± 0.02 | 270 ± 37.1 | 368.9 ± 3.72 | 5.72 ± 0.78 |
|           | 390 μatm CO₂ | 0.025 ± 0.005 | 0.50 ± 0.03 | 230 ± 34.9 | 368.9 ± 3.72 | 5.47 ± 1.32 |
|           | 800 μatm CO₂ | 0.017 ± 0.004 | 0.52 ± 0.01 | 229 ± 19.5 | 340 ± 36.1 | 7.5 ± 0.87 |
| -Fe       | 190 μatm CO₂ | 0.012 ± 0.001 | 0.33 ± 0.03 | 330 ± 26.1 | 368.9 ± 3.72 | 4.16 ± 0.40 |
|           | 390 μatm CO₂ | 0.013 ± 0.001 | 0.32 ± 0.02 | 301 ± 35.9 | 368.9 ± 3.72 | 5.29 ± 0.58 |
|           | 800 μatm CO₂ | 0.01 ± 0.001 | 0.32 ± 0.02 | 301 ± 35.9 | 368.9 ± 3.72 | 5.29 ± 0.58 |

Final Chl a: POC ratios (μg μg⁻¹) and photophysiological parameters (apparent maximum quantum yield of PSII F_v/F_m, proportion of non-photochemical quenching, NPQ, functional absorption cross section of PSII (μm² quanta⁻¹)), and relative electron transport rates from PSII (rETR) of the final communities grown under different CO₂ and Fe levels (n = 3; mean ± 1 s.d.). Fe-depleted (-Fe) and Fe-enriched (+Fe) conditions were achieved by the addition of 10 nmol kg⁻¹ DFB and 1 nmol kg⁻¹ FeCl₃, respectively. Bold p values indicate statistically significant differences between treatments (p < 0.05; 2-way ANOVA).

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Figure 2. Iron uptake capacities of final phytoplankton communities. Fe uptake capacities (pmol Fe (μmol POC)⁻¹ h⁻¹) of Fe-enriched (solid bars) and -depleted (open bars) final phytoplankton communities estimated from 24 h incubation with 1 nM ⁵⁵Fe as a function of pCO₂ [μatm]. Statistical analysis (2-way ANOVA) revealed significant differences between Fe-treatments (F = 62.217, p = 0.001) but not between CO₂-treatments (F = 1.205, p = 0.349).

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Figure 3. Net primary production of final phytoplankton communities. NPP (nmol C (μmol POC)⁻¹ h⁻¹) was estimated from 14C incubations over 24 h as a function of pCO₂ [μatm]. Black and grey bars indicate Fe-enriched (solid bars) and -depleted treatments (open bars), respectively (n = 3; mean ± s.d.). ANOVA analysis revealed a significant effect of pCO₂ levels as well as a significant interaction term of Fe and pCO₂ levels as a function of pCO₂ levels (F = 15.56, p = 0.001; F = 15.56, p = 0.001). P values indicate statistically significant differences between treatments (p < 0.05; 2-way ANOVA).

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Effects of Fe Limitation & OA on SO Phytoplankton
Fe concentrations decreased over the course of the experiment. This can be attributed to precipitation and absorption of colloidal iron in the absence of significant concentrations of Fe-binding ligands [40]. Fe limitation of phytoplankton in the DFB treatments was confirmed by significant differences in $F_{v}/F_{m}$ between Fe-enriched and -depleted treatments (Table 2; Figure 1 B). $F_{v}/F_{m}$ values of Fe-depleted treatments are comparable to those observed in naturally Fe-limited phytoplankton communities [41]. In line with previous findings on SO phytoplankton, also other physiological parameters like NPQ and rETR differed significantly between Fe treatments (Table 2, [42,43]). Moreover, the significantly higher Fe uptake capacity in Fe-depleted treatments likely reflects the induction of high-affinity uptake systems and/or the selection of phytoplankton communities with greater Fe affinities [21,22]. All of these observations confirm Fe limitation in the Fe-depleted treatments.

**OA response under Fe-enriched conditions**

The observed CO$_2$-dependent increase in primary productivity under Fe-enriched conditions (Figure 3) confirms that CO$_2$ fixation in SO phytoplankton can be limited by carbon supply under current CO$_2$ concentrations [14,16]. This hypothesis is further supported by the decrease in Chl a/POC ratios and the increase in rETRs with increasing pCO$_2$ (Table 2). Similarly, Ihlen et al. [44] observed ETR$_{max}$ in Fe-sufficient *Chaetoceros muelleri* to increase with increasing CO$_2$. These findings suggest that the Calvin cycle, the major sink of photosynthetic energy [9], is the rate-limiting step of photosynthesis under low pCO$_2$ levels. NPQ was not affected under high pCO$_2$, Fe-enriched conditions. Under the applied irradiance of 40 μmol photons m$^{-2}$ s$^{-1}$, however, NPQ values were generally very low (Table 2) suggesting that there is little requirement for dissipation of light. For one of

**Figure 4. Representative microscopy pictures of species composition of the final communities.** A, Fe-enriched 190 μatm (39±5% *Pseudo-nitzschia*, 43±4% *Fragilariopsis*); B, Fe-enriched 390 μatm (40±9% *Pseudo-nitzschia*, 42±12% *Fragilariopsis*); C, Fe-enriched 800 μatm (72±5% *Fragilariopsis*); D, Fe-depleted 190 μatm (55±16% *Pseudo-nitzschia*, 26±20% *Syedropsis*); E, Fe-depleted 390 μatm (51±15% *Pseudo-nitzschia*, 29±16% *Syedropsis*); F, Fe-depleted 800 μatm (78±2% *Syedropsis*).

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**Discussion**

**Confirmation of Fe limitation**

Dissolved Fe concentrations in Fe-depleted abiotic controls remained at initial concentrations, showing that experimental manipulations and CO$_2$ bubbling resulted in no significant Fe contamination (Table 1). In Fe-enriched abiotic controls, dissolved Fe concentrations decreased over the course of the experiment. Fe concentrations decreased over the course of the experiment. This can be attributed to precipitation and absorption of colloidal iron in the absence of significant concentrations of Fe-binding ligands [40]. Fe limitation of phytoplankton in the DFB treatments was confirmed by significant differences in $F_{v}/F_{m}$ between Fe-enriched and -depleted treatments (Table 2; Figure 1 B). $F_{v}/F_{m}$ values of Fe-depleted treatments are comparable to those observed in naturally Fe-limited phytoplankton communities [41]. In line with previous findings on SO phytoplankton, also other physiological parameters like NPQ and rETR differed significantly between Fe treatments (Table 2, [42,43]). Moreover, the significantly higher Fe uptake capacity in Fe-depleted treatments likely reflects the induction of high-affinity uptake systems and/or the selection of phytoplankton communities with greater Fe affinities [21,22]. All of these observations confirm Fe limitation in the Fe-depleted treatments.

| Taxonomic group               | Initial 190 μatm | Initial 390 μatm | Initial 800 μatm | +Fe 190 μatm | +Fe 390 μatm | +Fe 800 μatm | −Fe 190 μatm | −Fe 390 μatm | −Fe 800 μatm |
|------------------------------|-----------------|-----------------|-----------------|-------------|-------------|-------------|-------------|-------------|-------------|
| *Pseudo-nitzschia* cf. turgiduloides | 5 ± 5 40 ± 9 3 ± 3 | 55 ± 16 51 ± 15 < 0.5 | 190 μatm | 390 μatm | 800 μatm | 190 μatm | 390 μatm | 800 μatm |
| *Syedropsis* sp.             | 1 1 ± 0 1 ± 3 | 26 ± 20 29 ± 16 78 ± 2 | 800 μatm | 390 μatm | 190 μatm | 190 μatm | 390 μatm | 800 μatm |
| *Fragilariopsis* cylindrus   | 32 43 ± 4 42 ± 12 72 ± 5 | 3 ± 2 < 0.5 2 ± 1 | 190 μatm | 390 μatm | 800 μatm | 190 μatm | 390 μatm | 800 μatm |
| *Chaetoceros* cf. simplex    | 6 11 ± 0 10 ± 2 | 7 ± 2 | 800 μatm | 390 μatm | 190 μatm | 190 μatm | 390 μatm | 800 μatm |
| *Phaeocystis* antarctica     | 18 1 ± 0 2 ± 2 | 7 ± 2 6 ± 6 < 0.5 | 190 μatm | 390 μatm | 800 μatm | 190 μatm | 390 μatm | 800 μatm |
| Unidentified flagellates      | 9 3 ± 1 2 ± 2 | 4 ± 2 6 ± 3 6 ± 0 | 190 μatm | 390 μatm | 800 μatm | 190 μatm | 390 μatm | 800 μatm |
| *Ceratoneis* closterium      | 6 < 0.5 1 ± 0 1 < 0.5 | 1 ± 0 1 ± 1 | 190 μatm | 390 μatm | 800 μatm | 190 μatm | 390 μatm | 800 μatm |
| Dinoflagellates               | 6 < 0.5 1 < 0.5 | 4 ± 2 6 ± 3 6 ± 0 | 190 μatm | 390 μatm | 800 μatm | 190 μatm | 390 μatm | 800 μatm |
| *Pseudo-nitzschia* cf. turgida | 2 < 0.5 0.5 0.5 | 0.5 < 0.5 < 0.5 | 190 μatm | 390 μatm | 800 μatm | 190 μatm | 390 μatm | 800 μatm |
| *Fragilariopsis* kerguelensis | 7 < 0.5 0.5 0.5 0.5 | 0.5 < 0.5 | 190 μatm | 390 μatm | 800 μatm | 190 μatm | 390 μatm | 800 μatm |
| *Thalassiosira* sp.           | 3 < 0.5 0.5 0.5 | 0.5 < 0.5 0.5 | 190 μatm | 390 μatm | 800 μatm | 190 μatm | 390 μatm | 800 μatm |
| Large *Chaetoceros* sp.       | 2 < 0.5 0.5 0.5 | 0.5 < 0.5 0.5 | 190 μatm | 390 μatm | 800 μatm | 190 μatm | 390 μatm | 800 μatm |
| *Rhizosolenia* sp.            | 11 < 0.5 0.5 0.5 | 0.5 < 0.5 | 190 μatm | 390 μatm | 800 μatm | 190 μatm | 390 μatm | 800 μatm |
| *Thalassithrix* sp.           | 1 < 0.5 0.5 0.5 | 0.5 < 0.5 | 190 μatm | 390 μatm | 800 μatm | 190 μatm | 390 μatm | 800 μatm |
| *Guinardia* sp.               | < 0.5 0.5 < 0.5 0.5 | 0.5 < 0.5 | 190 μatm | 390 μatm | 800 μatm | 190 μatm | 390 μatm | 800 μatm |
| *Eucampia* sp.                | < 0.5 < 0.5 0.5 0.5 | 0.5 < 0.5 | 190 μatm | 390 μatm | 800 μatm | 190 μatm | 390 μatm | 800 μatm |
| *Ciliates*                    | < 0.5 0.5 0.5 0.5 | 0.5 < 0.5 | 190 μatm | 390 μatm | 800 μatm | 190 μatm | 390 μatm | 800 μatm |
| *Silicoflagellates*           | < 0.5 0.5 0.5 | 0.5 < 0.5 | 190 μatm | 390 μatm | 800 μatm | 190 μatm | 390 μatm | 800 μatm |

Species composition of the initial community (n = 1) and at the end of the experiment (% of total cell count; n = 3; average ± 1 s.d.).

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**Table 3. Microscopic cell counts.**

Effects of Fe Limitation & OA on SO Phytoplankton
the dominant species in the Fe-enriched treatments, *Fragilariopsis cylindrus*, a significant induction of NPQ was only found at irradiances larger than 200 μmol photons m⁻² s⁻¹ [43]. We can therefore conclude that even when photosynthesis was carbon limited under low pCO₂, the applied irradiance was too low to induce NPQ under Fe-enriched conditions and other pathways were operated as electron sinks (e.g. midstream-oxidases [9]).

The changes in physiological responses in Fe-enriched phytoplankton assemblages were accompanied by a pronounced shift in the species composition (Figure 4; Table 3) from *Pseudo-nitzschia* cl. *tangathaloides* under low and intermediate pCO₂ to *Fragilariopsis cylindrus* under high pCO₂ levels. Likely mechanisms for this floristic shift include species-specific differences in carbon acquisition [15,43], as well as pH-mediated differences in cellular physiology, e.g. changes in electrochemical membrane potentials and ion transport processes [46]. *Pseudo-nitzschia* has also been observed to dominate in bloom situations after Fe fertilization [7], where pH increases due to biomass build-up and drawdown of CO₂. This is in line with results from CO₂ manipulations on SO phytoplankton assemblages, which were dominated by *Pseudo-nitzschia* at high pH [16]. In a laboratory study under Fe-enriched conditions, growth and rETRs of *Pseudo-nitzschia subarctica* were unaffected by pCO₂, suggesting that this species shows little to no responses to CO₂ fertilization [15]. Our field experiment suggests that *Fragilariopsis cylindrus*, in contrast, benefited from increased pCO₂. Even though information of OA responses for this species is lacking, the related *Fragilariopsis kerguelensis* showed enhanced rETRs with increasing pCO₂ (S. Trimborn, unpublished data).

We thus speculate that *F. cylindrus* increased its photosynthetic activity under elevated pCO₂, thereby outcompeting the otherwise faster growing *Pseudo-nitzschia*. Under OA, also the relative abundance of *Chaetoceros* sp. increased by 50% (Table 3), which is consistent with previous findings on SO phytoplankton assemblages [16] as well as growth responses of *Chaetoceros debilis* to increased pCO₂ [15].

**OA response under Fe-limitation**

The observation that under Fe-limitation, productivity was not stimulated by increasing pCO₂ (Figure 3) may indicate that Fe acts as the main limiting factor suppressing the effects of other nutrients such as inorganic carbon. Alternatively, the apparent insensitivity of primary production to OA may arise from antagonistic physiological responses to pCO₂ and pH.

Under Fe limitation, elevated pCO₂ significantly increased rETRs and decreased NPQ, while the functional absorption cross section was not affected (Table 2). These results suggest a greater electron sink associated with the Calvin cycle and thus a decreased need for energy dissipation under OA [44]. It is also known that linear electron transport (LET) towards the Calvin cycle is not the sole sink for photosynthetic energy and that, depending on the ATP demand of the cells, alternative electron pathways can play an important role (e.g. Mehler reaction, MOX pathway, pseudocyclic electron flow around PSI) [9]. Increased pCO₂, however, is known to decrease photorespiration and/or the need for carbon concentrating mechanism (CCMs), which would lead to a decrease in cellular ATP demand [47]. The observed increase in rETRs in Fe-limited cells at high pCO₂ levels (Table 2) may therefore rather be linked to higher LET rates than to alternative electron pathways. The higher LET, enabled by the enhanced CO₂ fixation in the Calvin cycle, could counteract the generally greater need for photoprotection under Fe limitation [42,43]. This could explain the opposing CO₂ effects on NPQ under Fe-depleted and Fe-enriched conditions. The CO₂ effect, apparent in photophysiology, is potentially masked in primary production by co-occurring pH effects on Fe bioavailability. According to the observed decline in Fe bioavailability with decreasing pH [18,19], Fe-depleted phytoplankton would experience the greatest Fe stress under high pCO₂.

In this study, Fe-limitation was achieved by the addition of the chelator DFB. Even though DFB has been shown to form strong complexes with Fe and thereby decrease Fe availability by >90% [36], phytoplankton can still access DFB-bound Fe to some extent [48,49]. Since the phytoplankton assemblages in our DFB-treatments were strongly Fe-limited (as demonstrated by photo-physiology, Fe and C uptake), bioavailability of Fe must have been largely reduced. Fe bioavailability also seems to be slightly reduced with increasing pCO₂ (Figure 2), as has been observed in natural phytoplankton communities (i.e. without any added chelators) and in studies using different chelators such as EDTA and DFB [18,19,50]. Also, Maldonado et al. [49] suggest that the in-situ organic Fe-complexes observed in the SO have similar bioavailability compared to DFB. Although the chemical nature of in-situ organic Fe-binding ligands is not fully resolved, hydroxamate siderophores have been reported [51]. It is thus possible that at least some of the organically bound Fe exhibits a similar pH-dependent bioavailability as induced by DFB, and thus may allow for the extrapolation of our results to field situations. In order to study the bioavailability of Fe associated with in-situ Fe-binding organic ligands under OA scenarios, future experiments without added chelators should be conducted. As the nature of Fe-binding ligands remains largely unknown and can vary spatially [52,53], one should address the Fe bioavailability of various compounds (e.g., humic acids, saccharids, exopolymeric substances) that were reported to affect iron biogeochemistry [36,54,55]. Furthermore, organic ligands control the bioavailability and the physico-chemistry of trace metals in general [52,56]. As some of those (Co, Cd, Zn) are also essential for phytoplankton physiology (e.g. for the activity of the carbonic anhydrase) [57], joint measurements of other trace metals as well as their ligands would be desirable.

Although primary productivity was not sensitive to OA under Fe limitation, we did observe CO₂-dependent species shifts, with *Pseudo-nitzschia* sp. dominating under low and *Syndetopsis* sp. under high pCO₂ (Figure 4). The low abundances of *F. cylindrus* in Fe-depleted treatments probably reflect the rather high sensitivity of this species towards Fe limitation [43], which could be due to low Fe uptake capacities observed for this species [21]. In contrast, *Pseudo-nitzschia* has been shown to be an efficient user of Fe under limiting concentrations [58] and sporadic Fe input events [59]. Interestingly, the final proportion of *Pseudo-nitzschia* declined strongly with increasing pCO₂, irrespectively of the Fe status (Table 3), suggesting that its growth rates must have been significantly lower than those of the dominant species (*F. cylindrus* and *Syndetopsis* sp. under Fe-enriched and -depleted conditions, respectively). This observation is in line with a recent study on *P. pseudodelicatissima*, whose growth rates were not affected by OA under either Fe-depleter nor -replete conditions [50]. As *Pseudo-nitzschia* generally does not seem to benefit from increased pCO₂ levels ([15,16,50], this study), one could expect OA to have a negative effect on the abundances of this genus under on-going climate change. At present, nothing is known about the Fe and CO₂ requirements of *Syndetopsis* [60]. However, a possible appearance of *Syndetopsis* in phytoplankton assemblages or incubation experiments might have been overlooked in past studies, as their delicately silicified frustules are very prone to dissolution [61] and not distinguishable from *Pseudo-nitzschia* by light microscopy (Figure 4).
Our results clearly demonstrate a strong difference in CO$_2$-dependent community structure between Fe-enriched and Fe-depleted conditions (Figure 4). To explain these shifts, more information on species-specific differences in Fe requirements, uptake, as well as allocation strategies [21,62,63], and inorganic carbon acquisition [15,16] is needed for all dominant species.

Biogeochemical implications

The findings of our study suggest that the effects of OA on primary production and community structure are strongly modulated by the prevailing Fe concentrations. Our results, and those of others [16,64], indicate a potential stimulation of the biological pump as a result of increased pCO$_2$ in Fe-replete regions. Under Fe enrichment and increasing pCO$_2$ levels, we observed a shift from weakly silicified *Pseudo-nitzschia* towards more heavily silicified *Fragilariopsis*. *Pseudo-nitzschia* remineralizes quickly in subsurface waters [65], while *Fragilariopsis* is a more efficient vector of carbon export [12]. Thus, enhanced primary production, in concert with potentially higher export efficiencies, could lead to a stronger downward flux of organic matter in Fe-replete areas under OA.

The described feedback by ‘CO$_2$-fertilisation’, however, may not operate over the broad expanse of the Fe-limited SO. These regional differences in CO$_2$-sensitivity might be even more pronounced in terms of carbon export efficiencies, as under Fe-depleted conditions no functional shifts in species composition were observed. Here, all assemblages were dominated by weakly silicified species such as *Pseudo-nitzschia* cf. *turgiduloides* [54] or *Syneodiscus* sp. [60]. Frustules of both species are delicate and only preserved in shallow waters or under special circumstances such as large aggregation events in combination with anoxia [61,65]. Irrespective of their potential for carbon export, all species dominating our incubations are ecologically important [61,66,67]. Furthermore, both *F. cylindrus* and *P. turgiduloides* are not only characteristic sea-ice algae but also dominate phytoplankton assemblages in open waters [66,67]. In fact, most genera being characteristic for SO phytoplankton assemblages were present in initial and final phytoplankton assemblages (Table 3). Overall, species in the incubations resemble a mixture of typical open-ocean and sea-ice associated species [68–71]. Hence, our interpretations may not be restricted to sea-ice influenced habitats only.

Our results suggest that the potential ‘CO$_2$ fertilization’ effect critically depends on the availability of Fe, determining how strongly the biological pump will serve as a carbon sink in the future SO. Realistic projections of primary production and CO$_2$ sequestration thus remain difficult as long as scenarios for Fe input as well as its bioavailability to phytoplankton remain poorly constrained [10,72]. The results of this study furthermore highlight the need to assess combined effects of important environmental factors in order to understand and predict responses to single stressors such as OA. In this respect, irradiance levels should also be considered as a potentially interacting factor. Indeed, the level of energization has been shown to strongly influence the strength of phytoplankton responses to OA [73], suggesting that also the interaction between Fe and CO$_2$ availability could be modulated by light conditions. To thoroughly assess consequences of OA, multi-factorial perturbation experiments (including factors such as different Fe sources or grazing) should target physiological as well as ecological responses of SO phytoplankton assemblages.

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**Author Contributions**

Conceived and designed the experiments: CJMH ST BR CH PDT. Performed the experiments: CJMH ST CDP. Analyzed the data: CJMH ST CDP. Contributed reagents/materials/analysis tools: CJMH ST BR CH PDT. Wrote the paper: CJMH ST BR CH PDT. Wrote the revised manuscript: CJMH ST BR CH PDT. Wrote the response to the reviewers: CJMH ST BR CH PDT.

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