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Temperature-enhanced effects of iron on Southern Ocean phytoplankton

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Short summary

Phytoplankton growth in the Southern Ocean (SO) is often limited by low iron (Fe) concentrations. Sea surface warming impacts Fe availability and can affect phytoplankton growth. We used Fe clean shipboard incubations to test how changes in Fe and temperature affect SO phytoplankton. Their abundances usually increased with Fe addition and temperature increase, with Fe being the major factor. These findings imply potential shifts in ecosystem structure, impacting food webs and elemental cycling.

Abstract

Iron (Fe) is a key limiting nutrient for Southern Ocean phytoplankton. Input of Fe into the Southern Ocean is projected to change due to global warming, yet the combined effects of a concurrent increase in temperature with Fe addition on phytoplankton growth and community composition are understudied. To improve our understanding of how Antarctic phytoplankton communities respond to Fe and enhanced temperature, we performed four full factorial onboard bioassays under trace metal clean conditions with phytoplankton communities from different regions of the Weddell and the Amundsen Seas in the Southern Ocean. Treatments consisted of a combined 2 nM Fe addition with 2 °C warming treatment (TF), compared to the single factor treatments of Fe addition at *in-situ* temperature (F), and non-Fe addition at + 2 °C (T) and at *in-situ* temperature (C). Temperature had limited effect by itself but boosted the positive response of the phytoplankton to Fe addition. Photosynthetic efficiency, phytoplankton abundances, and chlorophyll *a* concentrations typically increased (significantly) with Fe addition (F and/or TF treatments) and the phytoplankton community generally shifted from haptophytes to diatoms upon Fe addition. The < 20 µm phytoplankton fraction displayed population-specific growth responses, resulting in a pronounced shift in community composition and size distribution (mainly towards larger-sized phytoplankton) for the F and TF treatment. Such distinct enhanced impact of Fe supply with warming on Antarctic phytoplankton size, growth and composition will likely affect trophic transfer efficiency and ecosystem structure, with potential significance for the biological carbon pump.

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1. Introduction

The Southern Ocean plays an important role in regulating the Earth´s climate as it is an important sink for CO_2 (Takahashi et al., 2012; Friedlingstein et al., 2022; Fisher et al., 2023). Phytoplankton take up CO_2 and convert it to biomass, forming not only the base of the pelagic food web but also driving the biological carbon pump (Buesseler et al., 2020; Huang et al., 2023). During the short austral productive season, however, Antarctic phytoplankton growth often becomes limited by low iron (Fe) availability (Martin et al., 1990; Boyd, 2002; Ryan-Keogh et al., 2023). Fe is a vital micronutrient for a variety of cellular processes, including photosynthesis (Geider & La Roche, 1994; Schoffman et al., 2016; Kroh & Pilon, 2020) and nitrate assimilation (Schoffman et al., 2016). Shortage of Fe results in so called high nutrient, low chlorophyll (HNLC) conditions, where the ratio of macronutrients, especially nitrate, relative to total Chlorophyll *a* (Chl *a*) concentrations is comparably high (Minas & Minas, 1992; Sarmiento et al., 2004; Venables & Moore, 2010; Basterretxea et al., 2023). Trace metal supply in the Southern Ocean follows a strong seasonal cycle where in winter Fe is replenished via deep water-mixing (Tagliabue et al., 2014) or sediment resuspension in coastal areas (Boyd et al., 2012), but this supply is quickly depleted again by phytoplankton uptake in the next season. However, Fe limitation for Antarctic phytoplankton is predicted to be at least partially relieved in the future (Bazzani et al., 2023) because of enhanced Fe supply by increased wind driven mixing (due to reduced ice-induced stratification) and sources associated with ice melt, i.e., glaciers (Annett et al., 2015; Sherrell et al., 2015; Van der Merwe et al., 2019; L. Seyitmuhammedov et al., 2022) icebergs (Raiswell et al., 2008; Shaw et al., 2011; Raiswell et al., 2016; Hopwood et al., 2019) or sea-ice (Lannuzel et al., 2016; Gerringa et al., 2020). These changes in Fe supply are associated with ongoing climate change that is projected to lead to elevated temperatures and changes in wind patterns as well as associated currents and upwelling (Turner et al., 2005; Moore et al., 2018). Overall, future Southern Ocean conditions will most likely be warmer with potentially elevated Fe concentrations, which can be expected to also affect phytoplankton productivity and community composition (Boyd et al., 2015; Laufkötter et al., 2015, Pinkerton et al., 2021). Considering the urgency of warming and the anticipated change in Fe supply, there is a need for studies investigating the combined effects of these two important drivers controlling phytoplankton growth in the Southern Ocean. There are many reports on the effects of Fe addition to Fe-limited phytoplankton from the Southern Ocean (Reviewed by e.g. Yoon et al., 2018; Bazzani et al., 2023) and several on the influence of temperature (Reay et al., 2001; Morán et al., 2006; Boyd et al., 2013), but only few studies examined the combined effects of Fe and temperature on Antarctic phytoplankton (i.e. Rose et al., 2009; Zhu et al., 2016; Andrew et al., 2019; Jabre & Bertrand, 2020; Jabre et al., 2021; Aflenzer et al., 2023). In particular, studies using natural

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phytoplankton communities are scarce (Rose et al., 2009; Jabre et al., 2021) and concentrated on Ross Sea phytoplankton with relatively large temperature increases (3 to 6 °C). Hence, more insight into how phytoplankton from other regional Antarctic seas respond to the warming projected by the year ~2100 (Meredith et al., 2019) is needed.

The Weddell Sea is one of the key areas of dense Antarctic bottom water formation (Fahrbach et al., 2004) and plays an important role in the global thermohaline circulation. The subpolar cyclonic Weddell Gyre circulating in the Weddell Sea basin isolates the centre of the Weddell Sea from marginal Fe sources such as melt or sediments, whilst the currents on the edges of the gyre have the potential to pick up Fe from a variety of sources, such as the seafloor (Klunder et al., 2014; Sieber et al., 2021, Tian et al., in prep.). Generally, the Weddell Sea has a relatively low primary productivity, associated with Fe limitation in the centre of gyre (Hoppema et al., 2007; Klunder et al., 2014). In contrast, the west Amundsen Sea and specifically the Amundsen Sea Polynya (ASP) is known as one of the most productive regions in the Southern Ocean in terms of net primary production per net area (Arrigo & van Dijken, 2003). Additionally, this region (ASP) is characterised by a fast thinning of ice-sheets, shelf ice and glaciers, with associated input of Fe required to sustain the high levels of primary productivity (e.g. Gerringa et al., 2012; van Manen et al., 2022). Nevertheless, phytoplankton in the ASP could still be stimulated by additional Fe input (Alderkamp et al., 2015).

The aim of the current study is to examine the concurrent effects of Fe supply and warming on Antarctic phytoplankton communities from the Weddell Sea and the Amundsen Sea under controlled trace metal clean conditions. Given the naturally low dissolved Fe (dFe) concentrations in the Southern Ocean, trace metal clean conditions are crucial to avoid confounding Fe effects when studying temperature alone (Middag et al., 2023). Our bioassay treatments comprised of Fe addition (F treatment), warming (T treatment), Fe addition and warming (TF treatment) and the control (no Fe addition, no warming; C treatment). The temperature was enhanced by 2 °C, based on forecasts from the IPCC report (Meredith et al., 2019). Whilst the Amundsen Sea has shown a warming trend over the past years already (Gómez-Valdivia et al., 2023; Drijfhout et al., 2024), the surface waters of the Weddell Sea might not increase as much with climate change, but show short-term local temperature increases (Darelius et al., 2023; Morrison et al., 2023; Teske et al., 2024). The concentration of dFe in the Fe addition treatments (F and TF) was increased by 2 nM. Future Fe concentrations are highly uncertain (Hutchins & Boyd, 2016; Tagliabue et al., 2016; Ryan-Keogh et al., 2023), and not necessarily linked to bioavailability of Fe (Van Manen et al., 2022; Fourquez et al., 2023), but previous experiments in the Southern Ocean have shown that such an addition represents (temporarily) Fe replete conditions (De Baar et al., 2005). Moreover, increased

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Fe availability in the Southern Ocean could have a far-reaching impact, leading to increased nutrient consumption consequently reducing nutrient transfer to lower latitudes where primary production is fuelled by these nutrients (Primeau et al., 2013; Moore et al., 2018). By integrating biological and trace metal chemistry analyses within large volume (20 L cubitainers), trace metal clean experiments, we aim to provide a clearer understanding of future changes in phytoplankton growth patterns and the implications for the Southern Ocean's role in global climate regulation.

2. Material and Methods

2.1 Location and sampling

Natural seawater for the bioassays was collected during research expeditions (Fig. 1) in the Amundsen Sea (bioassays A1 and A2, R/V Araon, ANA08B, 2017/18) and in the Weddell Sea (bioassays W1 and W2, R/V Polarstern, Alfred-Wegener-Institut Helmholtz-Zentrum für Polar- und Meeresforschung (2017), PS117, 2018/19) in austral summer (December – February).

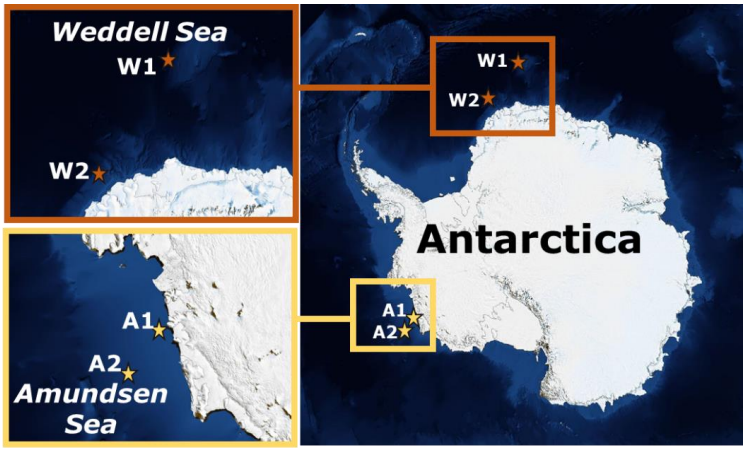

Figure 1: Location of the four bioassay experiments: Bioassays A1 and A2 were performed in the Amundsen Sea and W1 and W2 in the Weddell Sea (Image obtained from NASA Worldview).

Seawater was sampled at the autofluorescence maximum (36 m for A2 and 20 m for both W1 and W2), except for bioassay A1, which did not show an autofluorescence maximum and was sampled at the mid-mixed layer depth (15 m). Water for each bioassay was collected in a single deployment of NIOZ's Titan ultraclean CTD sampling system for trace metals (De Baar et al., 2008), mounted with pristine large volume samplers (Rijkenberg

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et al., 2015). To prevent light shock for the phytoplankton, the original PVDF Pristine samplers were replaced by a light-proof poly-propylene version. Salinity (conductivity), temperature, fluorescence, depth (pressure) and oxygen were measured with a CTD (Seabird SBE 911+) mounted on the trace metal clean sampling system (De Baar et al., 2008). To avoid contamination, further processing was performed under trace metal clean, dimmed light conditions and at 2 °C in a cleanroom environment inside a modified high-cube shipping container which fits the Titan sampling system.

Water for Amundsen Sea bioassay A1 was sampled from the middle of the Amundsen Sea Polynya (ASP) and for bioassays A2 in the marginal sea ice zone just outside of the ASP. Both W1 and W2 were performed with water from the eastern Weddell Sea. The Amundsen Sea bioassays A1 and A2 ran for 6 days (25 to 31 January and 31 January to 6 February 2018, respectively), whilst the Weddell Sea bioassays W1 and W2 ran for 8 days (28 December to 5 January and 9 to 17 January 2019, respectively). See Table 1 for *in-situ* environmental conditions at sampling depth (at the start of the bioassays). The *in-situ* temperature was below zero for all bioassays, with lowest values for A2 and W2 (-1.6 °C and -1.4 °C, respectively, compared to -0.6 °C and -0.3 °C for A1 and W1). The daily average irradiance at sampling depth on day of sampling was lowest for A1 and A2, i.e., < 6 µmol quanta m^{-2} s^{-1}, compared to 18 and 98 µmol quanta m^{-2} s^{-1} for W1 and W2.

Table 1: Characteristics of the seawater used for the bioassay experiments. Lat. = latitude, Long. = longitude, Temp = temperature, Si = silicate, PO_4 = phosphate, NOx = nitrate + nitrite, Fe = iron, Chl a = chlorophyll *a*, Phyto = total flow cytometry based phytoplankton abundances, F_v/F_m = photosynthetic capacity of the total phytoplankton. The reported irradiance is the average irradiance at the sampling depth on the day of sampling.

Bioassay	Station	Lat. (°S)	Long. (°W)	Temp. (°C)	Salinity (psu)	Irradiance (µmol quanta m^2 sec^{-1})	Si (µM)
A1	31	73.50	116.50	-0.6	33.99	5.0	84.7
A2	52	72.00	118.42	-1.6	33.89	3.1	78.5
W1	17	65.00	000.00	-0.3	33.90	17.7	58.3
W2	36	70.08	011.08	-1.4	33.82	97.6	27.7

Bioassay	PO_4 (µM)	NOx (µM)	Fe (nM)	total Chl *a* (µg L^{-1})	< 20 µm Chl *a* (%)	Phyto ($x10^3$ mL^{-1})	F_v/F_m r.u.
A1	1.8	27.7	0.28	3.0	42	8.4	0.6
A2	2.1	30.9	0.10	0.4	98	7.1	0.6
W1	1.6	24.0	0.05	1.5	24	5.6	0.3
W2	1.9	27.9	0.03	0.6	65	4.4	0.3

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2.2 Bioassay incubation set-up

Collapsible 20 L cubitainers (LDPE with PP caps and fitted with PE faucet; Cole-Palmer, Illinois, USA) were used for the bioassay incubations. These were soap and HCL (1 M) cleaned prior to the expeditions and stored with full surface contact in 0.024 M HCl (VWR Normatom Ultrapur, Avantor, Radnor, USA) for at least two months. Before use, cubitainers were rinsed five times with ambient seawater. The natural seawater for the actual incubations was distributed randomly to the total of 12 cubitainers which were then randomly assigned to the different treatments. Trace metal clean conditions were maintained during all sampling and sample handling.

The bioassay treatments (performed in triplicate) were: *in-situ* conditions (control, C), + 2 nM dFe (as $FeCl_3$) addition (F), + 2 °C temperature increase (T), and + 2 nM dFe addition and + 2 °C temperature increase (TF). For the Amundsen Sea bioassays, a natural isotopic composition (natural dFe) was used for the dFe addition, whilst $d^{57}Fe$ was used in the Weddell Sea bioassays. This practice was adopted to better differentiate the added Fe from the naturally present Fe, as we noticed that the dFe concentration in Fe amended Amundsen Sea bioassays quickly returned to background concentrations (see section 3.1). Measuring Fe with a natural isotopic composition at these low concentrations is still a challenge and combined with inherent variability between replicates. After several days it became impossible to distinguish the Fe amended and non-amended treatments in Amundsen Sea bioassays based on their natural dFe concentration (see section 3.1). The variation in natural dFe expected between Fe amended and non-amended treatments despite precipitation and uptake, was hidden in the analytical and environmental variability. For Weddell Sea bioassays we thus decided to add ^{57}Fe, a rare (2.12 % abundant *vs* 91.75 % for ^{56}Fe) natural isotope of Fe. Given its low natural abundance, ^{57}Fe is not nearly as sensitive to analytical and replicate variation as such variation is insignificant relative to the addition, allowing better insight in Fe drawdown over the course of the experiments.

Average starting concentrations of dFe in the Fe addition treatments ranged from 2.03 to 2.28 nM for both Weddell and Amundsen Sea bioassays. Temperatures in the T and TF treatments were 1.4, 0.4, 1.7 °C and 1 °C, for A1, A2, W1 and W2, respectively (see Table 1 for an overview of starting conditions in all treatments). One replicate of the control treatment in bioassay W1 started leaking during the incubation and was thus not sampled from day 4 onwards. For bioassay W2, the *in-situ* temperature of -1.4 °C could not be maintained due to the very sunny weather, resulting in an increase of 0.4°C for all treatments., Final incubation temperatures were -1.0 °C in the control (C) and Fe-only (F) treatment and 1 °C (instead of 0.6 °C) in the T and TF treatments. This temperature adjustment was done slowly over the course of 24 h on the second day of the incubation. More details about the

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set-up can be found in the supplemental data (Fig. S1, supplement Bioassay Setup). Over the course of the incubation period, temperatures were kept constant, with a maximum temperature fluctuation of ± 0.3 °C.

For Amundsen Sea bioassays, light levels were chosen to mimic *in-situ* conditions, but noting the low light conditions during these incubations (ca. 3 % of in-air photoactive radiation, PAR; i.e. average 3.4 and 1.5 µmol quanta m^{-2} s^{-1} for A1 and A2 over the course of incubation), we opted for non-limiting light conditions (Bertrand et al., 2011) for the (later performed) bioassays of the Weddell Sea (ca. 12 % of in-air PAR; i.e. average 69 and 100 µmol quanta m^{-2} s^{-1} for W1 and W2 over the course of incubation). The percentages and values reported refer to approximate light conditions within cubitainers. Samples for dissolved and particulate metals, Chl *a*, pigment-based taxonomic analyses, and particulate organic carbon (POC), nitrogen (PON) and phosphate (POP) were taken before filling of the cubitainers at the start of the bioassay incubations (t0), and at the end of the incubations after 6 (Amundsen Sea) or 8 (Weddell Sea) days. Samples for phytoplankton photosynthetic efficiency (F_v/F_m) and phytoplankton abundances were taken at least every other day. Macronutrients were measured on board at least every other day to screen for potential macronutrient limitation.

2.3 Setup verification

To test for potential Fe contamination, three cubitainers were filled with ultrapure (UP) water and handled and subsampled using the same methods and frequency as the treatments. Subsamples for dFe analysis were taken at the start (0.08 ±0.04 nM) and after three (0.07 ± 0.04 nM) and six (0.06 ± 0.04 nM) days. Concentrations of dFe stayed consistently low, suggesting minimal or no contamination. We also tested whether added dFe stayed in solution or adsorbed to the cubitainer walls and found a slow gradual decrease over the first few days in dFe concentrations after addition to UP water that we attribute to precipitation and wall adsorption (Table S1). During our experiments, the concentrations of added dFe decreased more rapidly, whereas the dFe concentrations in the non-Fe treatments, as well as the non-added form of dFe in Fe treatments ($d^{57}Fe$ for Amundsen and natural dFe for Weddell Sea bioassays), stayed low and relatively constant over time. Since phytoplankton grew in all treatments, the faster decrease of added dFe was likely due to uptake and sorption onto (biogenic) particles rather than precipitation to the cubitainer walls. Low traceable amounts of $d^{57}Fe$ during the second half of the incubations in W1 and W2 suggested that the initial decrease in Fe concentrations did not correspond to permanent removal from the bioavailable Fe pool (e.g. due to absorption; Jensen et al., 2020) but instead buffered the dissolved pool (as suggested for natural settings with exchange between the (labile) pFe and dFe pools; Van Manen et al., 2022),

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or that most of the added Fe was taken up by phytoplankton as rapid luxury uptake during the first days of an experiment (Lampe et al., 2018).

2.4 Macronutrients

During the Amundsen Sea bioassays, dissolved macronutrients were measured onboard following Jeon et al. (2021), according to the Joint Global Ocean Flux Study (JGOFS) protocols (Gordon et al., 1993) using a four-channel Auto-Analyzer (QuAAtro, Seal Analytical, Norderstedt, Germany). Measurement precisions were ± 0.02, ± 0.28 and ± 0.14 µM for phosphate, silicic acid, and nitrogen (nitrate + nitrite), respectively (Jeon et al., 2021). For Weddell Sea bioassays, samples for nitrate, nitrite, phosphate, and silicic acid were measured following the method described by Gerringa et al. (2019). Measurements precisions were ± 0.01, ± 0.31 and ± 0.04 µM for phosphate, silicic acid, and nitrogen (nitrate + nitrite), respectively.

2.5 Dissolved and particulate metals

Cubitainers were subsampled for dFe as well as other dissolved trace metals (dMn, dCo, dCu, dNi, dZn, dCd) using a 0.2 µm Sartobron-300 filter cartridge (Sartorius AG, Göttingen, DE) for bioassay A1 and A2 and pre-acid cleaned 0.2 µm Acropak filter cartridges (Cytiva, Marlborough, USA) for W1 and W2. Filters were fitted to an UP-cleaned vented PE faucet attached to the cubitainer with HCl acid (1.5 M) cleaned silicon tubing. Filtered samples were taken by applying pressure to the cubitainer. Different filters were used for Fe replete and deplete treatments, and filters were replaced between experiments. The dissolved trace metal samples were collected in acid cleaned 125 mL LDPE bottles following GEOTRACES protocols (Cutter et al., 2017) and directly acidified by adding ultra-pure HCl (Baseline®HCl; Seastar Chemicals Inc, Sidney, CA), resulting in a concentration of 0.024 M with a final pH of ~1.8. Samples were stored until analysis at NIOZ. Trace metal samples were prepared and analysed following van Manen et al. (2022) and references within. In short, trace metal samples were preconcentrated using a SeaFAST pre-concentration system (ESI). Blank contributions from sample handling, pre-concentration, and analysis steps were determined by analysing acidified MQ water (~1.8 pH) prepared in the same way as real samples.

For particulate trace metals (pMn, pCo, pCu, pNi, pZn, pCd) and POP, 25 mm poly-ether-sulfone (PES) disc filters (0.45 µm Pall Supor, Port Washington, USA) and polypropylene filter holders (Advantec, Cole-Parmer, Vernon Hills, USA) were used, following the protocol adapted by Van Manen et al. (2022) with one additional step: samples were soaked for at least 30 minutes in oxalate-EDTA (respectively 0.75M and 5.5M) in a 10L carboy

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(VWR Collection; Avantor, Radnor, USA) to remove all trace metals outside or adsorbed to phytoplankton cell walls (modified after Hassler & Schoemann, 2009) and subsequently filtered. Due to time limitations, samples for particulate metals were only taken during experiment A1, W1 and W2. Filters were stored frozen at -20 °C until analysis. In the NIOZ lab, filters were treated with two successive digestion steps to determine the total particulate fraction. All vials used in the digestion procedures were rigorously cleaned with HF and HCl beforehand and rinsed with UP water. Filters were subjected to a leach consisting of 1.8 mL of 4.35M (25 %) two times sub-boiled distilled acetic acid and 0.02M (2 %) hydroxylamine hydrochloride (99.999 % trace metal basis, Sigma-Aldrich, Saint-Louis, USA). Subsequently, filters were digested following the total digestion protocol developed by Cullen & Sherrell (1999) and modified by Planquette & Sherrell (2012). A volume of 2 mL of 3 × sub-boiled distilled 8.0 M (50 %) HNO_3 (VWR Chemicals – AnalaR NORMAPUR, Avantor, Radnor, USA) and 2.9 M (10 %) HF (Merck – Supelco, Kenilworth, USA) was added. The vials were closed tightly and refluxed for 4 h at 110 °C. The solution was then transferred to a secondary Teflon vial and were then heated to near dryness at 110 °C. A 1 mL volume of 8.0 M (50 %) 3× sub-boiled distilled HNO_3 (VWR Chemicals– AnalaR NORMAPUR, Avantor, Radnor, USA) and 15 % H_2O_2 (Merck – Suprapur, Kenilworth, USA) was added to the dried vial contents. The vials were refluxed for 1 h at 110 °C and subsequently cooled to room temperature. Addition of reagents and refluxing were repeated once. After this repetition, the vials were heated to near dryness at 110 °C. The samples were re-dissolved in 2 mL 1.5 % 3× sub-boiled distilled HNO_3 with 10 ppb Rh as internal standard and transferred to 2 mL Cryovials® (VWR, Avantor, Radnor, USA) for storage and analysis.

2.6 ICP-MS trace metal measurements and particulate organic phosphorous

Dissolved trace metal samples were preconcentrated using a SeaFAST pre-concentration system (ESI) using two loops of 10 mL and were eluted into 350 µL elution acid (1.5 M Teflon distilled HNO_3 with rhodium as internal standard) which gives a pre-concentration factor of 57.14 (see van Manen et al., 2022). Dissolved trace metal samples, blanks (Supplementary Table S2), and references (Table S3) were analysed by ICP-MS (Thermo Scientific Sector Field High-Resolution Element 2, Thermo Fisher-Scientific, Waltham, USA). Blank values were much lower than the analysed samples, and reference results were in good agreement with certified values.

For the particulate samples, including POP, the procedure blanks without a filter were treated identically to the samples, except for the steps involving filter handling and the removing of the filter from the filter holders. Therefore, the vial blank is included in this reagent blank. Filter blanks consisted of unused acid cleaned PES disc filters (Table S4).

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Accuracy and precision of the digestions were assessed by Certified Reference Materials (CRMs). There is no CRM available for marine suspended particulate matter, therefore accuracy could only be approximated by analysis of other available CRMs. PACS-2 and MESS-3 (marine sediments, National Research Council of Canada) were analysed. For each CRM, 10-30 mg were digested, whilst recommended sample weights are 250 mg for PACS-2 and MESS-3. The lower sample weights in this study were chosen to be representative of actual marine particulate suspended matter concentrations (similar to Ohnemus et al., 2014). PACS-2 and MESS-3 were only subjected to the total digestion (Table S5). The CRMs were in good agreement with the certified values.

2.7 Particulate organic carbon and nitrogen

For POC and PON sampling, 1 L of unfiltered seawater was collected from each cubitainer and stored in dark bottles (Nalgene, Rochester, USA) at 1 °C until further processing (within 4 h after sampling). Filtrations were then performed using combusted (4 h at 500 °C; Verardo et al., 1990) 0.3 µm 25 mm GF75 filters (Whatman, Cytiva, Maidstone UK) and under modest under pressure (max. 200 mbar). Filters were folded once, packed in aluminium foil, and stored frozen (-20 ºC) until analysis. The POC and PON concentrations were measured using a Thermo-Interscience Flash EA1112 Series Elemental Analyzer (Thermo Scientific, Waltham, USA) with excess oxygen, at 900 °C and a detection limit of 100 ppm and a precision of 0.3 % (Verardo et al., 1990). Before analysis, GF75 filters were folded and packed into a tin cup. The instrument blank is included by the analyser calibration. Carbon and nitrogen content of samples and blanks were computed according to the results of the standard measurements, and the blank was subtracted from the sample. Acetanilide (C_8H_9NO) with 71.09 % C and 10.36 % N (ThermoQuest, Milan, Italy) was measured as standard material, and silty and sandy soil standards from Elemental Microanalysis were measured as an internal reference.

2.8 Phytoplankton photosynthetic efficiency

F_v/F_m was determined in a Water-K quartz cuvette (3.5 mL) using pulse amplitude modulated fluorometry (Heinz Walz WATER-PAM, with Red LEDWATER-ED cuvette version S/N EDEE0196, Walz GmbH, Effeltrich, Germany). Samples were kept in 50 mL Greiner tubes (Thermo Fisher-Scientific, Waltham, USA) in the cold (stored in a cool box on ice) and in the dark for dark-adaptation (15 min up to occasionally 4 h). Acclimation times of up to 4 h did not affect photosynthetic efficiency of different phytoplankton (L. Peperzak, personal communication; Eich et al., 2021). The cuvette was rinsed with ultra-pure (UP) water between samples and the relative fluorescence yield (Ft) values were kept between 100 and 1000 by adjusting the PM-gain. Blanking was

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done for each station and/or bioassay using 0.2 µm filtered seawater from the respective stations and repeated after PM-gain adjustment when needed. The following formula was used to obtain the photosynthetic efficiency: $F_v/F_m = (F_m-F_0)/F_m$, with F_0 being the minimum fluorescence, and F_m being the maximum fluorescence.

2.9 Chlorophyll a concentration and pigment-based taxonomic analyses

Samples (0.54 - 2.65 L) for Chl *a* concentrations and pigment-based community composition were filtered within 30 min of subsampling (kept on ice and in the dark) on GF/F glass fibre filters (25 mm diameter, Whatman, Cytiva, Marlborough, USA) using a vacuum pump (max. 200 mbar), until filters showed clear colouring. Samples were taken for total as well as a < 20 µm fraction for better compatibility with phytoplankton community measurements by flow cytometry. For the < 20 µm fraction, natural seawater was reverse sieved through a 20 µm mesh before filtration onto a GF/F filter. Due to low sample volume availability at bioassay A2, the same amount of water from all replicates was combined for both total and < 20 µm Chl *a* samples, resulting in one averaged value for each treatment. Filters were folded once and double wrapped in aluminium foil, flash-frozen in liquid nitrogen and stored at -80 °C until further analysis in the home lab. Pigments were dissolved in 90 % acetone from the freeze-dried filters according to Van Leeuwe et al. (2006) and high-performance liquid chromatography (HPLC) pigment separation was performed (Zobrax-Eclipse XDB-C8 column, 3.5 µm particle size) according to Van Heukelem & Thomas (2001). Detection of pigments was based on both the retention time and diode array spectroscopy of standards (346 nm, Waters 996), quantification was based on calibration curves using those standards (DHI LAB standards). Phytoplankton community composition was determined using CHEMTAX version 1.95 (Mackey et al., 1996), following Selz et al. (2018). For the final pigment ratios, see Table S6.

2.10 Phytoplankton cell abundances (< 20 µm)

Phytoplankton cell abundances were obtained using a 488 nm Argon laser benchtop Beckton-Dickinson FACSCalibur (BD Biosciences, Franklin Lakes, USA) flow cytometer with the trigger set on red Chl *a* autofluorescence (Marie et al., 1999). The phytoplankton samples from the Amundsen Sea bioassays were measured fresh within 30 min of sampling (stored on ice); the Weddell Sea bioassay phytoplankton samples were fixed for 15 – 30 min with 100 µL formaldehyde-hexamine (18 % v/v:10 % v/v) at 4 °C, flash-frozen in liquid nitrogen and stored at -80 °C until analysis in the home lab. Phytoplankton populations were differentiated based on their red autofluorescence and side scatter, using FCS express 5 (De Novo Software, Pasadena, CA, USA). Freshly counted samples resulted in comparable gating as the fixed samples (tested for Amundsen Sea samples).

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A total of 25 populations were distinguished (Table S7), whereby not all populations occurred in both seas and all bioassays. Average cell diameters were determined by size-fractionation, i.e., serial gravity filtration through 20, 10, 8, 5, 3, 2, 1, 0.8 and 0.6 µm PC filters (Whatman, Cytiva, USA, Marlborough, MA) using a reuseable filter holder (Whatman, Cytiva, Marlborough, USA) and a plastic syringe. The number of cells retained by each filter per discriminated population were plotted against the respective filter size. The average cell diameters were defined as the size where 50 % of the original number of cells were retained, based on the fit of a sigmoidal plot (Veldhuis & Kraay, 2004). Phyto 5, 6, 7, 11, 12 and 14 were cryptophytes that were identified by their orange phycoerythrin autofluorescence. Based on earlier work (Biggs et al., 2019), we consider phytoplankton populations Phyto 20 and 22 to 25 to be diatoms and Phyto 8 to be *Phaeocystis antarctica* by comparing the red autofluorescence and side scatter pattern of the respective phytoplankton groups. The latter was confirmed during the Amundsen Sea expedition when we selectively collected *Phaeocystis* colonies and analysed them fresh onboard after gentle shaking (to break up the colonies). Phytoplankton carbon was estimated based on cell volume of phytoplankton, assuming spherical cells, and using 237 fg C μm^{-3} for picophytoplankton populations Phyto 1 to 6 and 196.5 fg C μm^{-3} for nanophytoplankton populations Phyto 7 to 25 (Garrison et al., 2000; Worden et al., 2004).

2.11 Statistical analyses

All statistical analyses were performed using R (R Core Team, 2021). To detect differences in phytoplankton community composition between treatments, an ANOSIM analysis was performed (vegan library, using Bray-Curtis dissimilarity with 9999 permutations). When a significant difference ($p < 0.05$) was detected, an indicator species analysis (vegan library, function r.g. with 9999 permutations) was used as a follow-up analysis to see which phytoplankton groups differed between treatments. This was done for both flow cytometry-based abundances and pigment-based taxonomic group composition, using relative values, thus normalized against total Chl *a* for pigment-based community composition, and total phytoplankton abundance for both pigment-based and flow cytometry-based phytoplankton groups. For the indicator species analysis, p-values are reported. A Scheirer-Ray-Hare test (non-parametric ANOVA-like test) was performed to determine the significance of Fe-addition and temperature increase, as well as potential interaction effects, on the respective response variable measured. The test was performed for data of the last day of the incubation, since effects were usually strongest then, and some variables were only sampled at the beginning and the end of the experiment (day 6 for A1 and A2, day 8 for W1 and W2). We manually calculated eta-squared (η^2, amount of variance explained, the higher the value, the larger

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the effect) by dividing the sum of squares of the effect of interest (i.e. iron addition, temperature increase and the interaction between these two) by the total sum of squares. The η^2 is provided when temperature increase, iron addition, and/or the interaction between both tested as significant. Since we wanted to look at the overall effect of Fe addition, temperature increase, and potential interaction effects on total phytoplankton abundances based on flow cytometry, we additionally performed a generalized linear model (GLM), assuming a quasi-poisson distribution in combination with a log-link, including the bioassay as well as the day number as factors without interaction, and including an interaction term for the Fe- and temperature-treatment. For the GLM, the data of all bioassays and all timepoints (excluding day 0) were combined. The formula for the GLM was: total phytoplankton abundances ~ Fe treatment * temperature treatment + bioassay name + day number. Statistical results are only reported for variables where more than 1 replicate was available. We also performed an NMDS analysis based on phytoplankton abundances using the vegan library with Bray-Curtis dissimilarity (seed set to 123). A significance level of $p < 0.05$ was used. Where applicable, the mean ± standard deviation is reported, unless stated otherwise.

3. Results

3.1 Nutrient dynamics

Bioassay treatments without Fe addition (C and T) started at naturally low dFe concentrations (0.28 ± 0.16, 0.10 ± 0.02, 0.05 ± 0.03 and 0.03 ± 0.01 nM natural dFe for bioassay A1, A2, W1 and W2, respectively), and stayed within these ranges. The Fe addition treatments (F and TF) showed a rapid (and overall comparable) drawdown of the added Fe (natural Fe for A1 and A2; Fig 2a, b and $d^{57}Fe$ for W1 and W2; Fig. 2e, f) in all bioassays, regardless of its isotopic composition. The dFe concentrations in F and TF treatments (0.29 ± 0.07 nM) at the end of the bioassays were comparable to concentrations in non-Fe addition treatments (0.28 ± 0.14 nM) for the relatively high-Chl *a* bioassay A1. In contrast, bioassay A2 had most dFe left at the end of the incubation (0.80 ± 0.46 nM for F and TF, compared to 0.11 ± 0.04 nM for C and T) which concurs with the low starting Chl *a* concentration and irradiance intensity. However, since the average dFe concentration in Fe amended treatments was lower (0.65 ± 0.10 nM) in the middle of the incubation period (day 3, see Figure 2 b), we cannot rule out potential contamination during sampling as a reason for the higher dFe concentrations, notably in the F treatment. For the Weddell Sea bioassays, $d^{57}Fe$ in F and TF treatments declined rapidly with low final concentrations (0.14 ± 0.03 and 0.31 ± 0.17 nM for W1 and W2, respectively) compared to the non-Fe addition treatments (0.01 ± 0.01 nM $d^{57}Fe$ and below detection limit for W1 and W2, respectively). Other trace metals were also measured, and dissolved manganese (dMn) drawdown did not differ between treatments (Fig. 2g-j). However, the starting

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concentrations of dMn were low for W1 and W2 (0.06 ± 0.03 and 0.19, SD < 0.01 nM, compared to 0.76, SD < 0.01 1.16 ± 0.01 nM for A1 and A2, respectively).

The dissolved inorganic macronutrients were not limiting phytoplankton growth during the bioassays. Final concentrations were at least 7.2, 0.3 and 37 µM in all bioassays for nitrogen, phosphate, and silicate, respectively (Supplement Fig. S2). Still, there was discernible drawdown of macronutrients by the microbial community during the incubations, except for Amundsen Sea bioassay A2. Fe addition (both F and TF treatments) had a significant impact on phosphorous drawdown for bioassays A1, W1 and W2 ($p < 0.05$, η^2: 0.53, 0.76 and 0.76 for A1, W1 and W2, respectively; and on average 0.45 µM lower for Fe addition treatments compared to C) and on nitrogen drawdown for bioassays W1 and W2 ($p < 0.004$, $\eta^2 > 0.75$, average of 9.8 µM lower for Fe addition treatments compared to C). The TF treatment showed stronger drawdowns especially for Weddell Sea bioassays W1 and W2 (average 0.7-fold change between TF and F treatments for both phosphorus and nitrogen, respectively), however there was no significant interaction effect between temperature increase and Fe addition. In contrast, silicate acid concentrations at the end of the incubation period were impacted by the increase in temperature for bioassays A1, A2 and W2 ($p < 0.02$, η^2: 0.76 for A1 and W2 and η^2: 0.52 for A2 and $p = 0.06$ and η^2: 0.32 for bioassay W1), with T treatments showing on average a 2.4 µM lower silicate concentration compared to the control. Only bioassay W1 showed an effect of Fe-addition on silicate drawdown ($p = 0.02$, η^2: 0.52), resulting in the TF treatment showing lowest concentrations on the last day of the incubations (0.8-fold change compared to the control and 0.9-fold change compared to both T and F treatment). The ratios of silicate drawdown to nitrogen and to phosphorus were higher in W1 than in W2 (i.e., 1.4 and 18.3 in W1 and 0.7 and 10.5 in W2). Moreover, when Fe was added, the silicate to nitrogen ratio (Si:N), as well as silicate to phosphorous ratio (Si:P) drawdown was lower in bioassays A1, W1 and W2 compared to non-Fe treatments (0.86 and 1.02 Si:N for Fe and non-Fe treatments and 11.3 and 12.5 Si:P, respectively).

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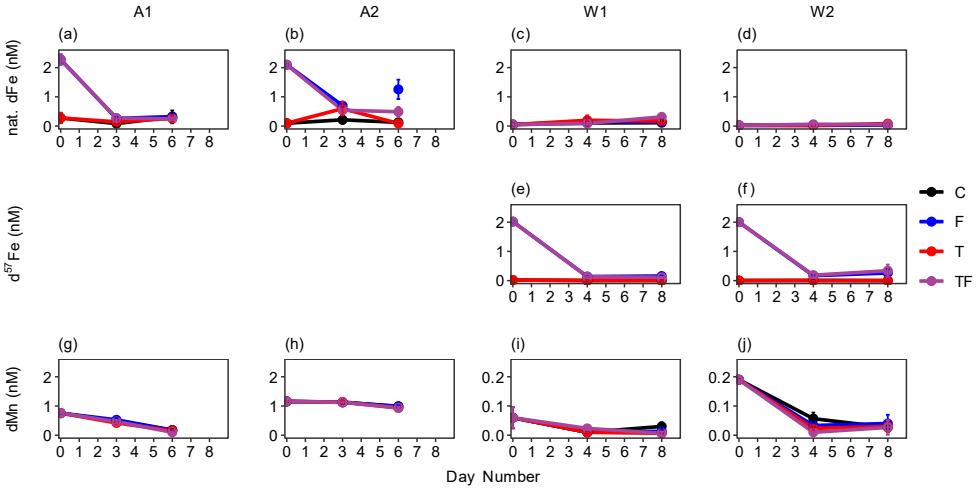

Figure 2: Average concentrations of natural dissolved Fe (a, b, c, d), $d^{57}Fe$ (e, f) and dMn(g, h, i, j) concentrations for Amundsen Sea (A1: a, g; A2: b, h) and Weddell Sea (W1: c, e, i; W2: d, f, g) bioassays. Amundsen Sea bioassays did not receive 57Fe supplementation. The black line represents the control (C) treatment, the red line the temperature (T) treatment, the blue line the iron (F) treatment, and the purple line the combined temperature and iron treatment (TF). Error bars indicates the standard deviation (n = 2 or 3, except for dFe of bioassay A2 TF treatment day 3, when they are not visible it is smaller than the symbol. Bioassay A2 showed a higher dFe concentration on day 6 compared to day 3, which we cannot exclude to be due to potential contamination and was thus treated as an outlier.

Particulate Fe concentrations (natural pFe for A1, $p^{57}Fe$ for W1 and W2) increased over time for the Fe addition treatments (Table S8) in all bioassays examined (excluding A2 as particulate metals were not measured there), and pFe concentrations at the last day of incubations were (positively) impacted by Fe-addition ($p \leq 0.01$, $\eta^2 \geq 0.73$ for A1, W1 and W2, final concentrations were 8.01 ± 0.83, 1.09 ± 0.10, 0.89 ± 0.33 nM for Fe addition treatments and 4.40 ± 0.21, 0.08 ± 0.02, 0.09, SD < 0.01 nM for treatments without Fe addition for A1, W1 and W2, respectively).

To examine potential differences in phytoplankton trace metal stoichiometry in response to Fe addition and/or warming, we calculated the ratio of pFe and other trace metals (pMn, pZn, pCd and pCu) to POP concentrations (Fig. 3, Table S8). Fe-addition significantly increased pFe:POP ratios (natural pFe for A1 and $p^{57}Fe$ for W1, W2) for all bioassays ($p \leq 0.01$, $\eta2 \geq 0.73$; average 2.5-fold change for natural pFe:POP (A1) and 13.3-fold change for $p^{57}Fe$:POP in Weddell Sea bioassays for Fe-addition treatments compared to the control). Furthermore, the pMn:POP ratios increased (by 0.33 compared to C) due to Fe-addition in bioassay A1 and decreased (by 0.13 compared to C) in W2 ($p < 0.01$ and 0.004, $\eta2$: 0.74 and 0.76, respectively). For bioassay W1, neither Fe nor temperature alone had a significant impact on the pMn:POP ratio, however, the combination of both treatments tested significant ($p = 0.01$, $\eta2$: 0.63), with the TF treatment showing an average 1.4-fold changed ratio compared to all other treatments. Also, the pCd:POP ratio was significantly affected by Fe-addition in W1 and W2 ($p < 0.05$,

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η2: 0.76 and 0.39 for W1 and W2), showing decreased values (by on average 0.12) for Fe-addition treatments compared to the control (Fig. 3 o-q), however no effect was seen for bioassay A1. A similar outcome was observed for pZn:POP ratios ($p \leq 0.01$, η2: 0.65 and 0.76 for W1 and W2, respectively, by on average 1.8 compared to C). For pCu:POP ratios, a decrease due to Fe-addition was mainly observed in bioassay A1 and W2 ($p < 0.009$, $\eta2 \geq 0.73$, by on average 0.16 compared to C), while for bioassay W1, Fe-addition caused a notable, but not statistically significant effect ($p = 0.09$, η2: 0.32, Fig. 3, by on average 0.23 compared to C).

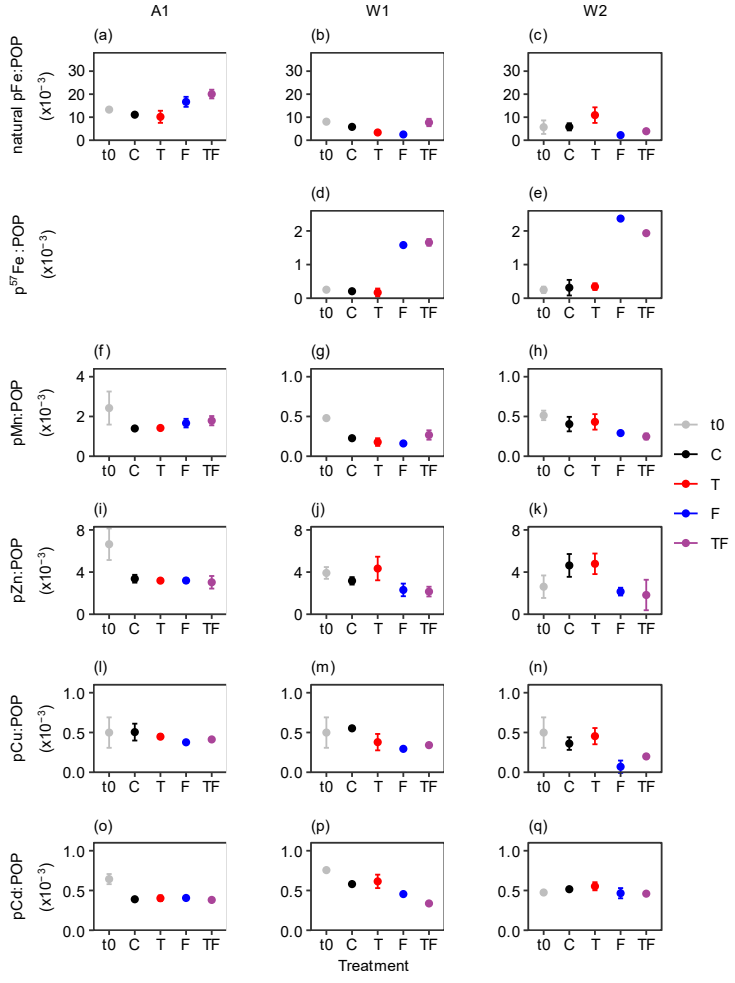

Figure 3: Average ratios (x10^{-3}, mM:M) of particulate trace metal to particulate organic phosphorus (POP) for Amundsen Sea A1 (a, f, I, l, o) and Weddell Sea W1 (b, d, g, j, m, p) and W2 (c, e, h, k, n, q) bioassays. There is no data available for A2. pFe = natural particulate, p^{57}Fe = particulate iron in the ^{57}Fe form (not added to Bioassay A1), pMn = particulate Manganese, pZn = particulate Zinc, pCu = particulate copper, pCd = particulate cadmium. t0 are starting ratios, whilst ratios for C (control), T (temperature), F (iron) and TF (combination of temperature and iron) were measured on the last day of the incubations (day 6 and 8 for Amundsen and Weddell Sea bioassays, respectively). Error bars indicates the standard deviation (n = 2 or 3), except for bioassay A1, T-treatment for all ratios and bioassay W1 C treatment for the pFe:POP ratio, there n = 1. If the error bar is not visible, then it is smaller than the symbol. Please note the different y-axis ranges for manganese to POP ratios (f–h).

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3.2 Photosynthetic efficiency

The photosynthetic efficiency F_v/F_m at the start of the incubations was 2-fold lower for the Weddell Sea bioassays compared to the Amundsen Sea bioassays (i.e., 0.3 *vs* 0.6 r.u., respectively). Fe addition led to an increase of F_v/F_m for all bioassays (Fig. 4, $p \leq 0.009$; $\eta^2 > 0.68$ for all bioassays), with stronger increases in Weddell Sea compared to Amundsen Sea bioassays (average of 1.42- and 1.14-fold change for Fe addition (F and TF) versus control treatments for Weddell and Amundsen Sea bioassays, respectively). Towards the end of the incubations of W1 and W2, F_v/F_m decreased slightly again for the Fe addition treatments (most so for TF, with final F_v/F_m values being still higher than for C and T treatments), coinciding with Fe depletion (Fig. 2).

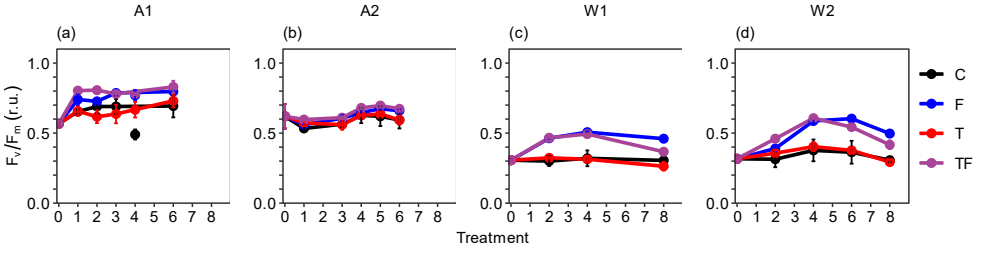

Figure 4: Temporal dynamics of the photosynthetic efficiency (F_v/F_m, relative units) of the phytoplankton for the Amundsen Sea A1 (A), A2 (B) and the Weddell Sea W1 (C) and W2 (D) bioassays. The black line represents the control (C) treatment, the red line the temperature (T) treatment, the blue line the iron (F) treatment, and the purple line the combined temperature and iron (TF) treatment. Averages of triplicates with error bars representing the standard deviation; if not visible it is smaller than the symbol. The control treatment of bioassay A1 showed an outlier for F_v/F_m values on day 4, which was excluded.

3.3 POC, Chl *a*, and phytoplankton taxonomic community composition

Total Chl *a* concentration at the start of the incubations (Table 1) was highest for the ASP bioassay A1 (3 µg L^{-1}) and lowest for bioassay A2 outside the ASP (0.4 µg L^{-1}). Of the Weddell Sea bioassays, W1 had the highest Chl *a* starting concentration (1.5 compared to 0.6 ug L^{-1} for W1 and W2). Starting concentrations of total POC in A1 and W1 were higher than A2 and W2 (384 and 347 ug L^{-1} compared to 91 and 136 ug L^{-1}, respectively). The POC to Chl *a* ratio was lower for A1 (130) than the other bioassays (212-239). Total POC concentrations did not display differences between treatments at the end of the incubations for A1 and A2 (Fig. 5a-d), yet total Chl *a* concentrations exhibited treatment-specific differences for all bioassays (Fig. 5e-h). Fe-addition always positively impacted Chl *a* concentrations (p: 0.02, 0.005 and 0.006, η^2: 0.52, 0.76 and 0.67 for bioassays A1, W1 and W2; not tested for A2 due to n = 1 for all Chl *a* samples and W1 C due to n = 1), however the effect was stronger in Weddell Sea Bioassays (average of 1.6- and 2.9-fold difference for Amundsen and Weddell Sea with Fe addition compared to C). Amundsen Sea bioassays also showed a slight increase in Chl *a* with increased temperatures.

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Strongest treatment-specific increases in Chl *a* concentrations were, however, obtained for the TF treatment in all bioassays, resulting in an average of 1.7 µg more Chl *a* L^{-1} compared to the F treatment. POC concentrations in W1 and W2 showed similar treatment responses as total Chl *a* in these bioassays.

The TF treatment also caused the strongest increase for the < 20 µm Chl *a* fraction (Fig. 5 e–h) for all bioassays, and Fe-addition generally had a positive impact on < 20 µm Chl *a* concentrations, with effects being strongest in both Weddell Sea bioassay W1 and W2 (increases of 1.2, 0.2, 0.5 and 0.7 µg L^{-1} for A1, A2, W1 and W2 compared to the control, respectively and $p = 0.04$ and 0.006, η^2: 0.37 and 0.67. A2 and W1 were not tested due to missing replicates). The < 20 µm fraction at the start of the bioassays made up respectively 42, 24 and 65 % of total Chl *a* in A1, W1 and W2, whereas for bioassay A2 95 % of the total Chl *a* concentration was < 20 µm. At the end of the bioassays, shares were 42, 25, 35 % and 70 % for A1, W1, W2 and A2, respectively.

Diatoms dominated the phytoplankton community at the start of A1 and W1 (53 and 62 % of total Chl *a*), followed by haptophytes (34 and 27 %; Fig. 5i-l). Bioassay W2 had a comparable share of diatoms and haptophytes (42 and 46 % of total Chl *a*), whilst the phytoplankton community of A2 was taxonomically most diverse.

Diatoms showed in general a strong response to Fe addition (F and TF treatment) and could be defined as an indicator group for Fe addition treatments in A1 and W2 ($p < 0.005$). Absolute diatom abundances increased as well with Fe-addition, especially for the TF treatment, in bioassays A1 (F and TF treatment, $p = 0.007$) and W2 (TF treatment, $p = 0.02$, Table S8). In bioassay W2, diatoms also showed a higher share for Fe addition treatments in the < 20 µm fraction ($p < 0.05$), with absolute abundances being higher in the TF treatment for bioassays A1, W1 and W2 ($p < 0.04$), and bioassay W1 also showing higher abundances at the F treatment ($p = 0.04$). The contribution of haptophytes declined (in response to the diatom increase, also in W1 where the diatom response was not significant, $p < 0.007$), however their absolute concentration (in µg Chl *a* L^{-1}; Table S8) did not decline except for the F-treatment in bioassay W2 ($p = 0.01$). Both the share ($p = 0.01$) and absolute concentration ($p = 0.04$) of pelagophytes increased with Fe addition in the < 20 µm fraction of bioassay A1. Cryptophyte abundances increased in the total fraction of the TF treatment for A1 and W2 ($p = 0.02$ and 0.01, respectively), and the < 20 µm fraction in W1 ($p = 0.02$), however their share did not change with treatments.

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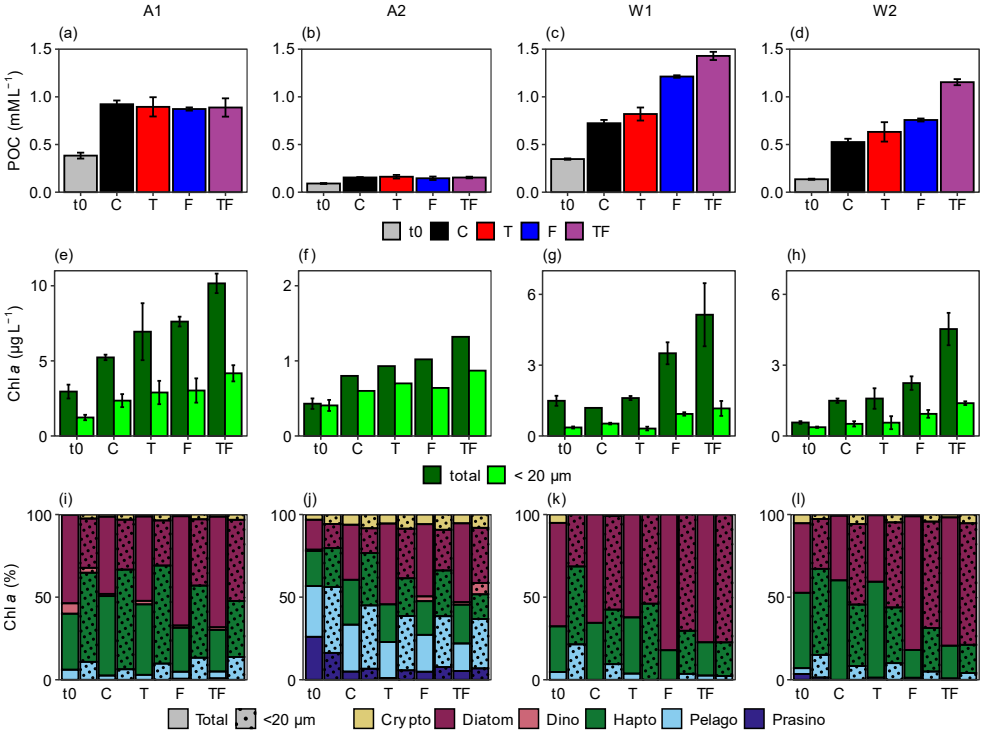

Figure 5: Average concentrations of particulate organic carbon (POC, a-d), total and < 20 µm (dotted columns) Chl *a* (e-h), and the taxonomic composition of the phytoplankton community (i-l , % of total Chl *a*) for the Amundsen Sea A1 (a, e, i) and A2 (b, f, j), and the Weddell Sea W1 (c, g, k) and W2 (d, h, l) bioassays. Error bars represent the standard deviation (n = 3 except when no error bar is shown, then n = 1). t0 = starting conditions, C = control, T = temperature treatment, F = iron addition treatment, TF = temperature and iron addition treatment. For i-l, Crypto, Dino, Hapto, Pelago and Chloro stands for cryptophytes, dinophytes, haptophytes, pelagophytes and chlorophytes, respectively. Solid bars represent the total and shaded bars the < 20 µm fraction community composition. Note the difference in y-axis for the Chl *a* panels e-h.

3.4 Phytoplankton abundances

The total abundances of < 20 µm phytoplankton (Fig. 6, Fig. S3, Table S8) increased with time for all bioassays and the treatment-specific dynamics largely mimicked the responses observed for the < 20 µm Chl *a* fraction (Fig. 5e-h). Bioassay A1 had overall the highest phytoplankton abundances (up to 40,000 ± 4,000 cells mL^{-1} for the TF treatment; Fig. 6a) and was dominated by *Phaeocystis antarctica* Phyto 8 (highest abundances of 37,053 mL^{-1} were observed in the TF treatment; Table S8). Phyto 19 increased specifically in abundance and share (Fig. 6a) in the temperature treatments (2,800 and 3.500 cells mL^{-1} for T and TF, compared to 1,700 and 1.300 mL^{-1} for C and F, $p < 0.01$). Phyto 24 was positively impacted by the TF treatment, i.e., 361 *vs* 595 cells mL^{-1} for C and TF treatment, respectively ($p < 0.05$).

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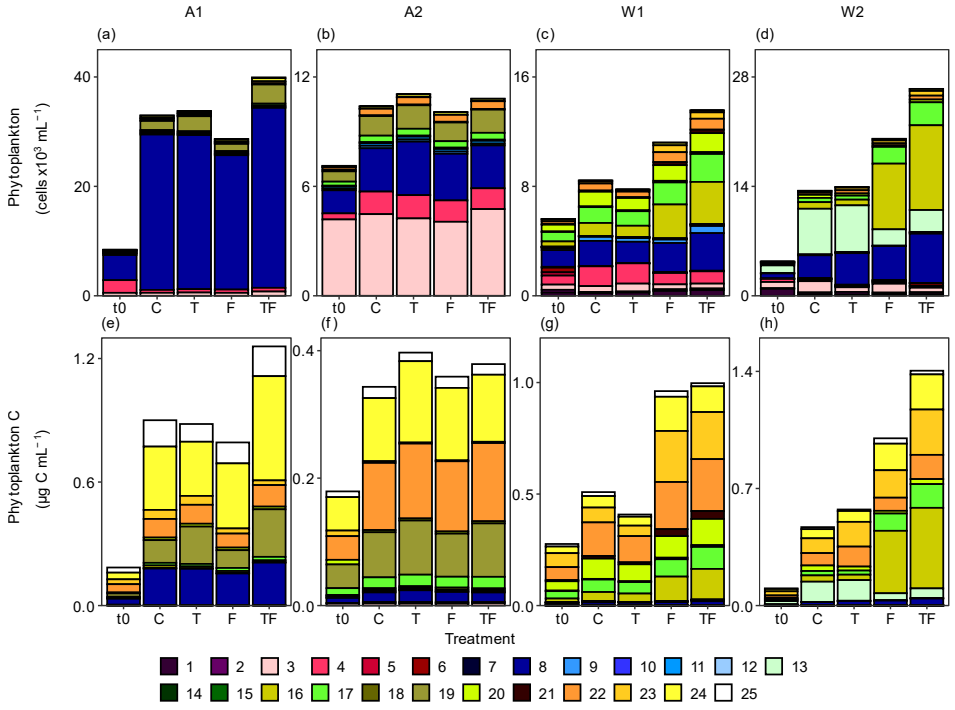

Figure 6: Flow cytometry-based phytoplankton community composition (a-d) and carbon (e-h) at the start (t0) and the end of the bioassay incubations for the different treatments (average of triplicates) for Amundsen Sea bioassays A1 (a, e), A2 (b, f), and Weddell Sea bioassays W1 (c, g) and W2 (d, h). t0 = starting conditions, C = control, T = temperature treatment, F = iron addition treatment, TF = temperature and iron addition treatment. Phytoplankton groups are sorted by size, with Phyto 1 – 6 ≤ 3 µm, Phyto 7 – 20 ≤ 10 µm and Phyto 21 – 25 ≥ 10 µm. Phyto 5, 6, 7, 11, 12 and 14 are cryptophytes, Phyto 20, 22 – 25 diatoms and Phyto 8 *Phaeocystis antarctica*. Note the different scales.

When converted to cellular carbon based on cell volume using 237 and 196.5 fg C μm^{-3} as conversion factors for Pico- and Nanophytoplankton, respectively (Fig. 6e), the strong positive response of the phytoplankton to the TF treatment was mostly due to this larger-sized Phyto 24 (average diameter of 19 µm, $p = 0.01$, stat: 0.92) and to smaller extent Phyto 19 ($p < 0.01$). Bioassay A2 presented the highest share of picoeukaryotes, especially Phyto 3 (59 % compared to max. 18 % in the other bioassays, Fig. 6b). No apparent treatment-specific responses were recorded, apart from Phyto 19 that increased somewhat with warming ($p = 0.04$). The phytoplankton populations in W1 were distributed more equally (Fig. 6c), with higher abundances of especially Phyto 16 and 17 for the Fe addition treatments ($p < 0.05$, most pronounced for TF with average abundances of 3,103 ± 1,290 *vs* 948 ± 218 cells mL^{-1} and 2,041 ±572 *vs* 1,158 ± 216 cells mL^{-1} for Phyto 16 and 17 in the TF *vs* C treatments, respectively). When expressed in carbon, Phyto 16 was still a recognisable indicator species ($p = 0.03$) but at the same time the larger-sized Phyto 21 (average cell diameter of 10 um) and diatoms Phyto 22-24 (13-19 µm) showed clear positive responses to Fe addition (Fig. 6g, $p < 0.05$ for all). Bioassay W2 also showed a distinct shift in favour of Phyto

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16 and Phyto 17 (away from Phyto 13), already early in time (Table S8), both for abundances and cellular carbon (Fig. 16d, h, $p < 0.01$ for all). Diatom 24 responded positively to Fe addition (F and TF, Fig. 16h, $p < 0.01$), similar as for bioassay W1, and diatom Phyto 23 showed higher abundances for the TF treatment ($p = 0.04$). Phyto 19 was the only phytoplankton population that showed a consistent selective positive response (in share) to warming (and not to Fe addition) in the Amundsen Sea bioassays. Diatom Phyto 22 increased with temperature in bioassay W2 ($p \leq 0.01$). We refer to Table S8 for less pronounced responses of the other phytoplankton populations. Overall, the response by the larger phytoplankton populations is also illustrated by the higher average cellular biovolumes in the F and TF treatments of W1 and W2 (Fig. S4). The Amundsen Sea bioassays did not show a treatment-specific increase in phytoplankton biovolume. Fe-addition had a significant effect on total phytoplankton abundances for Weddell Sea bioassays ($p < 0.02$, η^2: 0.96 and 0.74 for W1 and W2, with Fe addition leading to an average 1.6-fold change compared to C). The GLM we performed (explained deviance: 86 %), indicates an interaction effect of Fe-addition and warming ($p = 0.03$ for the interaction, exponentiated coefficient (ec): 1.13), i.e. Fe-addition of 2 nM in combination with a 2 °C temperature increase led to an overall increase in algal abundances of about 28 %. Fe-addition (ec: 1.03), temperature increase (ec: 1.11), bioassay and day number ($p < 0.001$ for all, for other statistical parameters, see Table S9) were also significant explanatory factors. The NMDS analysis of the Weddell Sea bioassays (Fig. S5c, d) demonstrated clear distinction between the Fe addition treatments and the non-addition treatments after the second day of the incubations. For bioassay W1, the TF and T treatments clustered on the last day of incubation separately from the F and C treatments, respectively. For bioassay W2, the T treatment also separated on the last day while the TF and F treatments remained closer together. Bioassays A1 and A2 did not display obvious clustering by treatment, other than time (i.e., separation after day 2).

4. Discussion

4.1 Trace metal and macronutrient dynamics

The pFe concentrations showed the expected significant increase in the Fe addition treatments for both Amundsen (natural dFe added) and the Weddell Sea bioassays ($d^{57}Fe$ added) at both temperatures, indicating that the added dFe was indeed taken up and incorporated in the phytoplankton cells. Additionally, in bioassay W2 the final $p^{57}Fe$ in the TF treatment was higher than in the F treatment (1.12 ± 0.11 nM compared to 0.66 ± 0.20 nM), demonstrating enhanced Fe uptake with higher temperatures. The higher starting concentrations of dFe in the Amundsen Sea, compared to the Weddell Sea, can be attributed to the Fe input from basal melt (Rignot et al.,

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2013). Conversely, the naturally low dFe concentrations in the Weddell Sea underscore the area's limited Fe input (e.g. de Baar et al., 1990; Klunder et al., 2012).

Fe is needed in nitrate assimilation and as such uptake of nitrate is coupled to the Fe nutritional status (Schoffman et al., 2016). Similarly, diatom cellular silicate to nitrogen ratios are known to increase in response to Fe stress (Meyerink et al., 2017). Highest drawdown of the macronutrients typically occurred in the TF treatment, which also showed the largest phytoplankton accumulation. However, whilst dissolved inorganic phosphate and nitrogen drawdown was mostly affected by Fe addition, silicate drawdown in bioassays A1 and W2 was more impacted by temperature. Despite a lower Chl *a* concentration (both total and < 20 µm) and phytoplankton abundance for the T than the TF treatment in these bioassays, the silicate drawdown was comparable. Although Fe stress is reported to cause reduced cellular Chl *a* concentrations compared to Fe replete conditions (Greene et al., 1992), it is an unlikely cause as the total phytoplankton abundances displayed similar differences between the T and TF treatment compared to < 20 µm Chl *a* concentrations. Instead, higher temperature may have stimulated Si uptake, as reported for the diatom *Pseudonitszchia seriata* at temperatures above 0 °C (Stapleford & Smith, 1996). It might also be that the T treatment experienced higher Fe stress than the control, which is also known to increase Si uptake (Meyerink et al., 2017). However, since phytoplankton abundances and Chl *a* concentrations were not higher in T treatments compared to the control, and since phytoplankton requires less Fe at higher temperatures (Jabre & Bertrand, 2020), this is less likely. Bioassay W1 showed the strongest decline in silicate concentrations, with both temperature and Fe affecting silicate drawdown. The relatively high fraction of diatoms (and specifically the large-sized Phyto 20 and 22-24) in bioassay W1 could theoretically have caused the strong silicate drawdown and high ratio of silicate relative to nitrogen (or phosphorus) uptake for all treatments. However, A1 also had high diatom abundances and over the course of the incubations the concentration of diatoms in W2 became comparable to W1. An alternative explanation may be that Mn stress in W1 (0.06 ± 0.04 *vs* 0.19 ± 0 nM in W1 and W2, respectively) enhanced Si uptake, similar to Fe stress (Hutchins & Bruland, 1998). Increased Si uptake by diatoms under a combined Fe and Mn limitation may possibly enhance protection against grazers (Assmy et al., 2013; Ryderheim et al., 2022) and/or enhance sinking to more nutrient-rich depths (Waite & Nodder, 2001). Considering an increasing awareness of trace metal co-limitation of phytoplankton growth (Wu et al., 2019; Browning et al., 2021; Balaguer et al., 2022; Burns et al., 2023), we recommend further investigation into these potential interactions and their ecological relevance.

Dissolved Mn is known to (co-)limit Southern Ocean phytoplankton growth and community composition (Balaguer et al., 2022). Although Fe addition (F and TF treatments) led to 1.8 (0.11 ± 0.03 *vs* 0.06 ± 0.02 d^{-1}) and

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1.5-fold (0.23 ± 0.02 *vs* 0.15 ± 0.01 d^{-1}) higher net growth rates (based on total phytoplankton abundances) in W1 and W2 compared to the control. The lower starting concentrations of dMn in W1 compared to W2 may have contributed to the 2-fold lower phytoplankton net growth rates in W1 compared to W2, independent of the treatment. Since Fe addition still led to an increased growth rate even with low dMn concentrations, Fe must have been the main limiting factor.

4.2 Micronutrient stoichiometry

The observed pFe:POP ratios increased upon the addition of iron (natural pFe for bioassay A1 and $p^{57}Fe$ for bioassays W1 and W2), validating the experimental design and confirming the uptake of added dFe by phytoplankton. For other bio-essential (Mn, Zn, Cu) or bio-active (Cd) metals, the metal:POP ratio is expected to be elevated under Fe stress due to upregulation of non-specific divalent metal transporters under Fe stress (e.g. Kustka et al., 2007; Lane et al., 2008) or the increased uptake of phosphorous relative to metals under Fe replete conditions (growth-dilution; Cullen et al., 2003). Specifically for Mn, this might also be due to a higher cellular Mn requirement under Fe stress (Peers & Price, 2004). The pMn:POP ratios were indeed higher in the C and T treatments compared to the F and TF treatments of W2, but for W1, no consistent effect of Fe was observed (Fig. 3).

In contrast, slightly elevated pMn:POP ratios were observed after Fe addition in A1 (F and TF treatments), matching findings by McCain et al. (2021) and Hawco et al. (2022), showing increased Mn demand in environments with high Fe concentrations. Such variation likely reflects adaptive changes in nutrient uptake and storage mechanisms under nutrient stress but could also be due to different phytoplankton community compositions and/or environmental conditions. For example, Twining et al. (2004) observed elevated pMn:POP ratios in individual diatom cells under iron deplete conditions, relative to iron replete conditions, whereas the trend was opposite for autotrophic flagellated cells. However, diatoms were dominant in the F and TF treatments in all experiments, suggesting that other factors besides differences in community composition play a role. The starting dMn concentrations differed between the bioassays, whereby the high starting concentrations of dMn in A1 could potentially explain the increased pMn:POP ratios in the F and TF treatments of this experiment., We speculate that a high availability of both Fe and Mn under the low light conditions in A1 could have led to increased Mn uptake for use in photosynthesis. Since dMn levels are thought to increase with Fe input (e.g. due to ice shelf melting; Van Manen et al., 2022), we recommend including dMn in future studies examining the effects of global climate change on phytoplankton growth.

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Besides Mn, other trace metals are known to have variable ratios with respect to POP under different environmental conditions. For example, cellular Cu requirements increase under Fe limitation (Schoffman et al., 2016), which could explain the higher pCu:POP ratios in the C and T treatments compared to the Fe addition treatments (Fig. 3). Similarly, the pZn:POP ratios were also elevated in the non-Fe treatments in W1 and W2, akin to the pCd:POP ratios especially in W1, suggesting higher uptake of metals under Fe limitation as previously suggested (Cunningham & John, 2017). Future studies linking these stoichiometric ratios with molecular measurements (e.g. protein expression patterns) could provide further insight into the processes which underpin trace metal uptake and use within phytoplankton communities under change. Nevertheless, this study highlights a potential trend of increased uptake of essential and non-essential metals (specifically zinc, copper and cadmium) by phytoplankton under Fe-deplete conditions. This trend underscores the adaptive strategies employed by phytoplankton in navigating nutrient scarcities, emphasizing the complexity of nutrient interactions and their collective impact on phytoplankton ecology under varying environmental conditions (e.g. Cunningham and John, 2017). Due to the importance of nutrient uptake in the Southern Ocean for the stoichiometry of global nutrient distributions, notably at lower latitudes (Sarmiento et al., 2004; Middag et al., 2020), changes in (micro-)nutrient consumption in the Southern Ocean can have global ramifications for both productivity and ecosystem structure (Moore et al., 2018) which should be further explored in future (modelling) studies.

4.3 Impact of Iron and Temperature on Phytoplankton Dynamics

The Weddell Sea bioassays exhibited stronger Chl *a* accumulation and increased phytoplankton abundances in response to Fe addition than the Amundsen Sea bioassays, which is likely due to the lower dFe concentrations (and hence higher degree of Fe limitation for the phytoplankton typical for the Weddell Sea) at the start of the incubations. While for the Weddell Sea bioassays the POC concentrations followed comparable responses to total Chl *a* upon Fe addition (Fig. 5), the POC concentrations in the Amundsen Sea bioassays did not. The lower irradiance during the incubations of A1 and A2 most likely led to the higher Chl *a*:POC ratios at the end of incubations (i.e., average over all treatments 0.008 ± 0.002 and 0.003 ± 0.003 for the Amundsen and Weddell Sea bioassays). Enhanced Chl *a*:POC ratios are a known acclimation to low light (Laws & Bannister, 1980; Geider, 1987; Geider et al., 1998; Wang et al., 2009). Despite the low light intensities, Chl *a* concentrations and phytoplankton abundances in the control treatment increased over time in the Amundsen Sea bioassays (especially in A1, net growth rate based on abundances of 0.23 ± 0.02 d^{-1}), which indicates that the phytoplankton communities were low light adapted. Low light conditions are common for Amundsen Sea phytoplankton

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(Schofield et al., 2015; Park et al., 2017) but still, the very low irradiance in A2 seemed to have limited growth (0.09 ± 0.01 d^{-1}) as also illustrated by incomplete depletion of the dFe added (after 6 days of incubation). Although earlier studies reported positive responses of phytoplankton to Fe addition also under low light conditions (Viljoen et al., 2018; Alderkamp et al., 2019), the light intensities used for the low light treatment in those studies were relatively high (i.e., 15 and 30 µmol quanta m^{-2} s^{-1}) and well above those in A1 and A2 (average of 3.4 and 1.5 µmol quanta m^{-2} s^{-1}). Considering diatoms being the taxonomic group responding strongest to Fe additions (Noiri et al., 2005; Feng et al., 2010; Hinz et al., 2012; Mills et al., 2012; Zhu et al., 2016), the low proportion of diatoms at the start of A2 may also have delayed a measurable effect of Fe addition. Since both Weddell Sea and Amundsen Sea bioassays were initiated at times corresponding to the peak phytoplankton growth periods in each region, it is unlikely that the sampling time had a major effect on our results.

Consistent with the lower dFe concentrations was the reduced *in-situ* F_v/F_m of the phytoplankton in W1 and W2 compared to Amundsen Sea bioassays, which stayed low for non-Fe addition treatments throughout the experiments. Although we cannot exclude that the lower light availability in A1 and A2 may have caused enhanced F_v/F_m (compared to W1 and W2; From et al., 2014), low F_v/F_m is a common indicator of Fe stress in the Southern Ocean (Greene et al., 1992; Olson et al., 2000; Mills et al., 2012; Jabre and Bertrand, 2020). In addition, the low dMn concentration at the start of bioassay W1 may have contributed to the low F_v/F_m (Wu et al., 2019). Nevertheless, Fe addition also had a positive effect on F_v/F_m in Amundsen Sea bioassays, matching earlier reports that F_v/F_m of ASP phytoplankton is partly controlled by Fe (Alderkamp et al., 2015). The decrease in F_v/F_m in the F and TF treatments towards the end of the Weddell Sea bioassays might indicate that added Fe depleted towards potentially limiting conditions or might be an indication of Mn (co-)limitation.

4. 4 Enhanced responses to Fe with warming

Fe addition led to an overall positive response of Chl *a* concentrations, phytoplankton photophysiology and growth, but more so when combined with the ecologically relevant increase in temperature. The increase in phytoplankton abundances was especially distinct for Weddell Sea bioassays. GLM analysis revealed that temperature alone was a significant factor for total phytoplankton abundances, however more specifically, only Phyto 19 and Phyto 22 abundances displayed significant positive responses to temperature alone (T treatment). The 2 °C warming alone was thus not a major driver of phytoplankton net growth in our bioassays, but accelerated and enhanced Fe-addition responses (significant interaction effect for iron addition and temperature increase on total phytoplankton abundances). The enhanced response to Fe with temperature was especially distinct for

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bioassay W2 (average 1.61-fold change in the TF treatment compared to both F and T treatments). Despite low light levels, this was also seen in Amundsen Sea bioassay A1, albeit to a lower extent (average 1.29-fold change in the TF treatment compared to both F and T treatments). Larger-sized (> 20 µm) diatoms were mainly responsible for the Chl *a* accumulation, which is consistent with previous studies (Noiri et al., 2005; Feng et al., 2010; Hinz et al., 2012; Mills et al., 2012; Zhu et al., 2016) and supports the general consensus that especially large phytoplankton show enhanced growth upon Fe addition due to their lower surface to volume ratio (Scharek et al., 1997). But also (slightly) smaller diatoms Phyto 23 and 24 (average cell diameter of 15 and 19 µm, respectively) responded positively to the combination of Fe and temperature. Diatoms Phyto 24 was even the main phytoplankton population responsible for the increase in the < 20 µm Chl *a* fraction of the TF treatment in A1. The NMDS analysis based on < 20 µm phytoplankton abundances showed clustering for W1 and W2 driven by Fe addition and temperature, indicating that also smaller-sized phytoplankton display positive responses. This is supported by increased < 20 µm Chl *a* concentrations and the 2.2 fold change in cellular carbon of < 20 µm phytoplankton in F and TF treatments in the Weddell Sea bioassays (compared to the C and T treatments). Specifically, we recorded distinct abundance increases of the small 7 µm Phyto 16 and Phyto 17, in the F and TF treatments of W2. *Phaeocytis antarctica* (Phyto 8; 3.7 µm) also displayed higher abundances under the TF treatment for W2 but the effect was not very apparent and overall, *P. antarctica* seems to handle the other treatments consistently well. Rose et al. (2009) and Zhu et al. (2016) also found diatoms preferentially stimulated by Fe addition and/or temperature over *P. antarctica*, which was also found in a broader context where *P. antarctica* dominated under Fe-low conditions, whilst diatoms dominated in regions with higher Fe concentrations (Arrigo et al., 2017). In contrast, Andrew et al. (2019) found comparable growth rates for *P. antarctica* and several diatom cultures (tested under Fe addition and warming treatments). Similar to our study, they found highest growth for the combined Fe addition and warming treatment for most species. Since diatoms tended to increase strongest with Fe addition, it can be speculated that phytoplankton community compositions shift towards more diatoms with increases in Fe concentrations, however other biogeochemical factors are also important to consider. The positive phytoplankton growth responses were population specific and Phyto 13 (5.5. µm) in W2 even showed reduced abundances for the F and TF treatments, underscoring the multifaceted factors controlling phytoplankton dynamics and emphasizing the importance of understanding how trace metal concentrations and climate change influence the marine ecosystems in the Southern Ocean.

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5. Conclusions

Our study stands out that it combined chemistry and biology, Chl *a*, and population abundance to examine co-effects using natural Antarctic phytoplankton communities at environmentally realistic Fe concentrations (+ 2 nM) and a predicted (2 °C) temperature increase (Boyd et al., 2015; Jabre et al., 2021; Andrew et al., 2022). So far, studies investigating combined effects using natural Antarctic phytoplankton communities focussed on the Ross Sea and tested 3 – 6 °C warming (Rose et al., 2009; Jabre et al., 2021). Our bioassays incubations were performed under trace metal clean conditions (the entire duration) and with temperature remaining stable over the course of incubations (maximum fluctuation of temperature ± 0.3 °C). We stress the importance of trace metal clean working conditions to avoid inadvertently assigning Fe addition effects on phytoplankton to temperature when working in low Fe regions (i.e. Southern Ocean, but also open oceans in general). The differences we found between the F and TF treatment may have been assigned to temperature alone under non-trace metal clean working conditions (as Fe would inadvertently have been introduced), whilst our results show that temperature alone did not have a (major) effect.

In general, the addition of Fe was the primary factor for observed stimulatory effects. In particular, large diatoms benefitted from Fe addition, although several smaller-sized phytoplankton populations showed enhanced abundances upon Fe addition. Given that the intensity of the observed effects varied between the experiments with distinctly different phytoplankton communities, this study emphasizes the need for studying diverse regions of the Southern Ocean to better understand and predict potential future changes, especially as future changes in Fe availability are region-specific (Tagliabue et al., 2016; Van Manen et al., 2022). Factors such as the dFe concentrations, other trace metal concentrations which may potentially co-limit phytoplankton growth, and light availability, should also be considered when studying the effects of future climate on Antarctic phytoplankton. Moreover, the time of the year affects the starting composition of the phytoplankton community, and the sequence of the treatments in case of dual treatments (Fe addition and temperature increase) may affect the responses (Brooks and Crowe 2019), hence these factors should be considered.

Depending on the geographical region and the time in the productive season (Thomalla et al., 2023), global warming is predicted to increase wind-induced mixing or strengthen vertical stratification (Bronselaer et al., 2020; De Lavergne et al., 2014; Hillenbrand & Cortese, 2006; Shi et al., 2020). Phytoplankton will bloom earlier in the productive season as a result of decreasing sea ice and consequently higher light (Krumhardt et al., 2022), rapidly drawing down available Fe, followed by stratification, and thus favourable conditions for smaller-sized phytoplankton (Deppeler & Davidson, 2017; Krumhardt et al., 2022). Our study shows, however, that enhanced

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Fe input in such regions may partly overturn this warming-induced shift, assuming macronutrients will not become limiting.

Alterations in phytoplankton community composition and cell size, as observed in our experiments, will directly affect top-down control by grazers and viral infection and consequently trophic transfer efficiency. Moreover, not only the flow of organic carbon through the food web will be affected, but also the flux of organic carbon to deeper layers of the ocean (biological carbon pump) depends on the phytoplankton community composition, cell size and type of loss factor. We recommend that future bioassay studies consider phytoplankton gross growth, grazing, and viral lysis as well. After all, the typically reported net changes in the bioassay phytoplankton community are the resultant of production and losses. Both Fe as well as temperature can impact the extent of the loss factors. For example, grazing rates are known to increase with temperature (Chen et al., 2012; Caron & Hutchins, 2013), viral lysis may occur faster at higher temperature (Maat et al., 2017), and Fe availability can affect algal virus production and infectivity (Slagter et al., 2016). Finally, potential region-specific differences in the share of grazing and lysis (Mojica et al., 2016; Mojica et al., 2021; Eich et al., 2022) may influence net changes in phytoplankton biomass or abundances in bioassays. The Southern Ocean biogeochemical cycling and ecosystems dynamics are complex and need to be better studied in field and modelling studies. The current study underlines the need for assessing consequences of near future temperature changes at environmentally relevant dFe concentrations.

Data availability: All data presented in this paper (nutrients, trace metals, phytoplankton abundances, photosynthetic efficiencies, Chl *a* concentrations, pigment based community composition, particulate organic carbon and particulate organic phosphate) are included in this published article and are available under https://doi.org/10.25850/nioz/7b.b.hh.

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Author contributions: RM and CPDB conceptualized the study. CE, MvM, EB, SBEHP, HT, IA, JSPM and RM conducted the fieldwork under the lead of RM, JJ analysed the nutrient samples for Amundsen Sea bioassays, WvdP analysed the pigment samples and conducted the Chemtax analysis, CE, MvM, wrote the original draft, CPDB and RM edited the paper. EB, LJ and JSPM contributed to the discussion. All authors contributed to commenting on the paper.

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