### We thank the reviewer for their valuable feedback. Below you find our detailed response (in blue italic).

### General comments:

A comprehensive examination of iron and temperature modulation of natural phytoplankton communities was conducted through two sets of bioassay experiments in two different regions of the Southern Ocean, namely the Amundsen Sea and the Weddell Sea, over the summer period. The experimental design was well-planned and executed with consideration of future climate change predictions. The resulting data and supporting information are structured and comprehensively presented in the manuscript. The future recommendations further enhance the relevance of this research and the propensity for continued investigations into related fields by highlighting several knowledge gaps.

### Detailed and technical comments:

I have outlined some suggested changes to consider for each section, as well as some minor technical corrections. My main suggestion would be that some statements in the discussion could benefit from including more recent publications where relevant to confirm or explain the results. Additionally, the conclusion could further be finessed to enhance clarity and impact.

We will include the suggested references to the Discussion and will change the Conclusion to be more concise (see our response to your Conclusion-specific comment later on).

#### Introduction

The introduction adequately highlights the knowledge gaps and thus provides the rationale for the study on temperature-enhanced effects of iron on the natural phytoplankton community in the different Southern Ocean regions. However, the results and discussion additionally introduce other parameters which could have been briefly included as part of the literature in the introduction. *We will add to the Introduction line 77 onwards: "Besides Fe and temperature, there are also other factors, e.g., other bio-essential metals (Mn, Co, Ni, Cu and Zn) where notably, Mn has been shown to be (co-)limiting in the Southern Ocean (Wu et al; 2019, Browning et al., 2021, Balaguer et al.; 2022, Hawco et al.; 2022). Mn is essential for phytoplankton photosystems (Raven et al., 1990) and a co-factor for enzymes dealing with oxidative stress (Wolfe-Simon et al., 2005). Moreover, light is another major limiting factor for phytoplankton growth in Southern Ocean (e.g. van Oijen et al.; 2004, Strzepek et al.; 2019, Vives et al.; 2022, Latour et al.; 2023)."* 

Line 60: The references listed for 'phytoplankton growth often becomes limited by low iron (Fe) availability' are some examples of the many existing publications. Rather indicate that these are some references ('e.g.') to the magnitude of studies that have indeed established that phytoplankton growth becomes limited by low Fe in different ocean basins.

The Reviewer is correct and we will add an "e.g.," to these references.

-Line 62: Consider adding 'Milligan and Harrison, 2000' for the 'nitrate assimilation' reference (https://onlinelibrary.wiley.com/doi/full/10.1046/j.1529-8817.2000.99013.x). *We thank the Reviewer for the reference and will add it accordingly.* 

-Line 70: Another reference to consider for 'Fe supply by increased wind-driven mixing' is 'Moreau et al. 2023' (<u>https://www.nature.com/articles/s41467-023-36992-1</u>). *We thank the Reviewer for the reference and will add it.* 

-Line 71: Typo 'L.' in the citation: 'L. Seyitmuhammedov et al., 2022'. *We fixed the typo.* 

-Line 92: Could you expand a little on the 'Fe from a variety of sources' apart from the seafloor?

We will add the underlined part to the sentence in line 92: "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, <u>bathymetry driven mixing with deeper water</u> <u>masses, and sources associated with ice melt</u> (Raiswell et al., 2008, Geochemical transactions, Vol 9; Shaw et al., 2011, Deep Sea Research Part II: Topical Studies in Oceanography, Vol 58 (11-12); Klunder et al., 2014, Biogeosciences, Vol 11 (3); Annett et al.; 2015; Sherrell et al., 2015; Lannuzel et al., 2016; Raiswell et al., 2016, Biogeosciences, Vol 13 (13); Hopwood et al., 2019; Van der Merwe et al., 2019; Gerringa et al., 2020; Sieber et al., 2021, Earth and Planetary Science Letters, Vol 567; Seyitmuhammedov et al., 2022; Tian et al., in prep.)."

### Materials and Methods

The materials and methods section was succinct. The section on the setup verification is much appreciated to remove any doubt of contamination issues, particularly for incubations performed while out at sea.

Figure 1: I would have appreciated seeing some information on the hydrography of the sampling sites, or even the Chla distribution in the map (separately). However, I understand that it is not so trivial, given the different sampling timelines.

We will add a depth profile for each station to the Supplements, as well as Chl a data based on NASA worldview at the time of sampling.

### -Line 141: What was the average PAR under the 'dimmed light conditions?

We did not measure the PAR in the clean container, however, the samplers are light proof and cubitainers were covered with black opaque bags to avoid light stress when transported on deck. We will add to line 193 in the Material and Methods section 2.2: "During transport on deck, cubitainers were covered with black light-proof bags to avoid light stress."

-Line 147: '28 December 2018 to 5 January 2019' *We will change the text accordingly.* 

-Line 153: Table 1:

- In the methods you refer to 'silicic acid'. Ensure it is clear that silicic acid is indeed the reported 'silicate'. If not, make this clear.
- It should be clearer if 'Fe' refers to dissolved Fe (dFe) only.
- Chl *a* (italicize a)

Silicic acid is indeed the reported silicate, we will change the text accordingly and be consistent with the names. We use dFe when referring dissolved iron only, and Fe when talking about iron in general, and will check that we stay consistent in this.

-Line 204: Could you please clarify what threshold/range is 'consistently low'? The ranges measured are given in lines 201 – 203 of the original manuscript. Concentrations within one standard deviation of the mean starting values (0.12 nM) were considered consistently low.

-Line 298: Section 2.8: Limited information on the instrument operation for the photophysiological data acquisition is given. Was any form of post-processing conducted on the raw Fv/Fm data? Or was this not necessary based on the data acquisition from the PAM?

No post-processing was necessary on the raw  $F_v/F_m$  data, and we will add more information on the measurement specifics.

-Have you considered investigating the effective absorption cross-section ( $\sigma$ PSII) from the photophysiological results? Could it further support the outcomes and contextualize the results in terms of stress on the photosystem or help estimate the primary production rates?

# Unfortunately, the instrument used does not permit for us to calculate the effective absorption cross section.

-Line 305: Perhaps you could reference 'Cullen and Davis, 2003' for the choice of 0.2  $\mu$ m filtered blank corrections (Cullen JJ, Davis RF (2003) The blank can make a big difference in oceanographic measurements. Limnol Oceanogr Bull 12:29–35)?

We thank the reviewer for the reference and will add it.

#### **Results:**

In the introduction, you highlighted that 'Generally, the Weddell Sea has a relatively low primary productivity', while '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'.

Based on these statements, it would be nice to see a brief contrast of the initial conditions, as well as the results obtained in these two areas. This may also be a precursor to a concluding statement about the differences in the temperature-enhanced effects of the phytoplankton from these two regions.

In the abstract, I missed specific outcomes that are expected to be different for these two regions We are hesitant to put too much emphasis on regional differences, given that we only performed two experiments in each region, and given that light conditions differed. We will, however, add a section on initial differences between the bioassays in the Results.

### "3.1 Sample site characteristics

The in-situ temperature was below zero for all bioassays, with lowest values for Amundsen Sea bioassay A2 and Weddell Sea bioassay 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  $\mu$ mol quanta m<sup>-2</sup> s<sup>-1</sup>, compared to 18 and 98  $\mu$ mol quanta m<sup>-2</sup> s<sup>-1</sup> for W1 and W2. Dissolved inorganic macronutrient concentrations were relatively comparable between bioassays, except the silicate concentration in W1 being  $\sim$ 20  $\mu$ M lower than for the other bioassays (but still far from limiting). Initial dFe concentrations in the Weddell Sea were lower compared to the Amundsen Sea (Table 1), as were dMn concentrations (Fig. 2). Bioassay A1 had the highest Chl a concentrations (sampled within the ASP), followed by W1. Both bioassays also had the highest share of >20  $\mu$ m Chl a. The Chl a concentration of A2 was almost exclusively made up of < 20  $\mu$ m sized phytoplankton (98% of total Chl a, Table 1). Flow cytometry derived phytoplankton abundances were highest for the Amundsen bioassays. The photosynthetic efficiency Fv/Fm 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). The station for bioassay W2 was closest to the coast, followed by A1, A2 and W1, however distance to land did not seem to have a major impact on either phytoplankton community composition, or nutrient concentrations."

-Line 483 and elsewhere: When referring to significant differences in Chla concentrations from bioassays, the Chla concentrations are a useful baseline. However, Chla growth rates are nuanced for assessing significant differences in phytoplankton responses from the bioassay experiments. Thus, it might be useful to reassess the significant differences in Chla by evaluating their growth rates instead. You already mentioned elsewhere Chla growth rates, but I did not easily find how this was calculated.

The net growth rates mentioned in the manuscript are based on total phytoplankton abundances, not on Chl a. Phytoplankton growth rates are based on an exponential trend line per replicate for each treatment and each bioassay, which we will add to the Material and Methods section. For Chl a, we unfortunately had limited time points, preventing proper rate calculations.

-Line 420: Figure 2: Typo: 'Weddell Sea (W1: c, e, i; W2: d, f, j)'? Thank you for noticing the typo, we will change the figure legend.

-The red and purple lines blend too well with each other. Consider a darker shade of purple or another colour. Apply comments to other similar figures. *We will adapt the figures accordingly.* 

-Line 423: missing ')' after 'day 3'? *We will add the missing bracket.* 

-Line 451: Figure 3: Typo: 'Amundsen Sea A1 (a, f, i, l, o)? Thank you for noticing the typo, we will change the figure legend.

-Line 470: Figure 4: '(a), (b), (c) and (d)' – change to lowercase to be consistent. We will change this.

-Line 109-110: Since short-term local temperature increases can be expected in the Weddell Sea, can you comment on the short-term temperature increase effects from your bioassays in W1 and W2? Would using lower incubation temperatures for this region be justifiable instead? *Temperature alone did not have a major effect on phytoplankton in the Weddell Sea, thus the short-term small temperature increases (Darelius et al., 2023) as such are unlikely to have a large, direct impact. However, if such temperature increase occurs in a period of time when dFe concentrations are high(er), we could expect some restricted local and shorter-term) phytoplankton responses (growth, increase in Chl a concentrations and POC). When these short-term increases occur on top of general warming, it may cause a (small) response by the phytoplankton. Still, we expect mostly an indirect effect from associated freshening (Darelius et al., 2023).* 

-Line 399: I missed how the nutrient drawdown was calculated. It would be nice to see a summary table or figure for the nutrient drawdown and the Chla drawdown, respectively. It is confusing to follow the results otherwise.

We did not calculate nutrient drawdown rates but compared nutrient concentrations at the start and end of the incubation period as nutrient drawdown, and additionally compared end concentrations between treatment to determine stronger and/or weaker nutrient drawdowns. We will add total drawdowns to the Supplements.

-Line 406: 'Silicate acid' or just 'silicate'? Check consistency in the use of terms throughout the manuscript.

It should indeed be silicate. We will change this throughout the manuscript to be consistent.

-Line 551: potential typo: '(Fig. 6d,h, p<0.01 for all).' And (F and TF, Fig. 6h, p < 0.01)? Thank you for noticing the typo, we should indeed be referring to Figure 6, not 16.

Discussion:

Line 608: 'Dissolved Mn is known to (co-)limit Southern Ocean phytoplankton growth and community composition (Balaguer et al., 2022).'

Mn is known to (co-)limit together with? Are these co-limitations necessarily seasonal? Consider: Pausch, et al. 2019.

(https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0221959)

Latour, et al. 2023. (https://online.ucpress.edu/elementa/article/11/1/00022/197210/Seasonality-of-phytoplankton-growth-limitation-by)

We thank the Reviewer for the references. We will edit the following section (following line 607; changed sections are underlined for easier following): "Dissolved Mn is known to (co-)limit Southern Ocean phytoplankton growth and community composition <u>together with Fe</u> (Browning et al. 2021, Balaguer et al. 2022). <u>Under such conditions, dFe addition alone positively impacts Chl a</u> <u>concentrations, phytoplankton abundances and POC concentrations, but a combination of dFe and</u> <u>dMn addition can lead to higher increases in these variables (Pausch et al. 2019, Browning et al., 2019)</u>

2021). Nevertheless, dMn addition effects can often be masked by the effects of dFe addition (Latour et al. 2023), and dFe addition alone can already lead to increases in ChI a even in primarily Mn-limited areas (Browning et al., 2021). This fits our results showing increases in in ChI a concentrations with dFe addition. Also net growth rates based on total phytoplankton abundances showed increases (i.e. 1. 5 ( $0.20 \pm 0.05 vs 0.12 \pm 0.02 d-1$ ) and 1.4-fold ( $0.24 \pm 0.01 vs 0.18 \pm 0.01 d-1$ ) higher for Fe-addition treatments (F and TF) compared to the control for bioassays W1 and W2. 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. <u>Our data</u> indicate potential dMn/dFe colimitation in the Weddell Sea already in early summer. Since the requirements for dMn and dFe differ between different phytoplankton groups (Arrigo, 2005; Twining & Baines, 2013; Balaquer et al., 2023), we suggest that the (co-)limitation of dMn and dFe may be affected by phytoplankton community composition. Considering that Mn limitation can be seasonal (Latour et al., 2023), we also urge to study different stages of the phytoplankton bloom period."

-Line 635: Typo: '.,'

Thank you for noticing, we will change the text accordingly.

# -Line 637: Is this increased Mn uptake consistent with the needs of the phytoplankton community found at A1?

Given that diatoms are highly abundant in the Fe addition treatments (both F and TF), and it is known for diatoms that their dMn demand increases with dFe addition (McCain 2021 Raven 1990, Hawco et al. 2022), we do think that the increase in Mn uptake is consistent with the needs of the phytoplankton community for those treatments in bioassay A1. However, diatoms are also known to have an increased Mn demand under Fe limitation (Peers & Price 2004). There is evidence (Hawco et al. 2022) for an increased Mn demand with increasing dFe concentrations for both a diatom- and a nanophytoplankton based model, suggesting that the increased Mn uptake observed for bioassay A1 is indeed consistent with the needs of the phytoplankton community. We will add the following to the Discussion on pMn:POP: "This duality in the pMn:POP ratios is not surprising as Mn demand may not only increase under Fe stress, but it should also increase with Fe addition, as both Mn and Fe are required for photosynthesis (Raven 1990, McCain 2021, Hawco et al. 2022). Hence, in an environment with low Mn concentrations, Fe addition can consequently lead to Mn limitation (e.g., Hawco et al., 2022). Mn concentrations at the start of bioassay A1 were relatively high, and indeed pMn:POP ratios increased with Fe addition, while concentrations of Mn decreased during the experiment. However, the low (potentially phytoplankton growth limiting) Mn concentrations in Weddell Sea bioassays from the start might have prevented a noticeable positive effect of Fe addition on Mn uptake. The higher biomass and cell abundance after Fe addition in these experiments implies the community had to make due with less Mn per cell than in the treatments without Fe addition (likely resulting in relatively low Mn auota despite elevated demand), potentially explaining why there was an increase in the pMn:POP ratios in the C and T treatments of W2, whereas this was not observed in W1 with even lower Mn starting concentrations."

# -Line 664: The average Chl a:POC ratio over all treatments for the Weddell Sea bioassays were 0.003 $\pm$ 0.003. Could you comment as to why the Chl a content is very low relative to the POC? Is this because of a significant variability in this ratio across different treatments?

The significant difference between treatments is only partly explaining the low ChI:POC ratios for the Weddell Sea. Fe addition did have a significant (positive) impact on ChI:POC ratios for Weddell Sea bioassays, however these ratios are still lower compared to the Amundsen Sea bioassays. Ratios were 0.003 and 0.004 for the F and TF treatment in both W1 and W2, with ratios for the C treatment being 0.002 and 0.003 for W1 and W2, respectively (no difference between C and T treatments). In contrast, ChI:POC ratios for Bioassays A1 were 0.006, 0.008, 0.009 and 0.011, and for Bioassay A2 0.005, 0.006, 0.007 and 0.009, for the C, T, F and TF treatments, respectively. As mentioned in line 665 in the original manuscript, we assume that ChI:POC values were higher in the Amundsen Sea as an adaption to low light. Moreover, the higher ChI:POC ratios in the Amundsen Sea (and consequently the lower

ratios in the Weddell Sea) might also be a sign for the difference in Fe limitation (and possibly Mn limitation) between the Weddell Sea and Amundsen Sea bioassays, since Fe-limited cells are known to have a lower ChI:POC ratio compared to non-limited and/or replete cells (Moore et al. 2007). We will add to line 666 in the original manuscript: "The relatively low ChI:POC ratios in the Weddell Sea bioassays (average over all treatments  $0.003 \pm 0.003$  vs  $0.008 \pm 0.002$  for the Amundsen Sea bioassays) may indicate stronger Fe limitation, since Fe limited cells are known to have a lower ChI:POC ratio compared to non-limited cells (Moore et al. 2007)."

-Line 673: The bioassays conducted by Viljoen et al. (2018) were in the Weddell Sea, while Alderkamp et al. (2019) conducted their bioassays in the Ross Sea. The bioassays presented in this manuscript were conducted in both the Amundsen Sea and Weddell Sea. The sampling season and location of W1 coincided with bioassay 'S54–65' by Viljoen et al. (2018). However, no comparisons seem to have been made or conclusions drawn regarding the outcomes based on similar and variable initial conditions to the overall outcomes from the bioassays.

Instead, only the 'low light conditions' where the light intensities differed due to Sea regions between this manuscript and Viljoen et al. (2018) as well as in Alderkamp et al. (2019) were highlighted.

We thank the Reviewer for pointing out that bioassay S54-65 by Viljoen et al 2018 was performed at a similar/closeby location as bioassay W1 and will add a comparison of these specific bioassays to our discussion: "The location the seawater for bioassay W1 was taken has similar coordinates as bioassay S54-65 in a study by Viljoen et al. (2018). These authors sampled 3 weeks later (different year) and at a comparable depth (30 m vs 20 m in our study) and found largely similar responses by the phytoplankton to dFe addition, i.e., total Chl a increased by  $\sim 2 \mu g$  Chl a L<sup>-1</sup> and diatoms dominated the phytoplankton community. In contrast to W1 but comparable to our other bioassays, total Chl a concentration in bioassay S54-65 (Viljoen et al. 2018) increased in the control over the duration of the bioassay. The lack of increase in Chl a in the control (and T) treatment of W1 might be explained by a lower in-situ dFe for W1, indicating a stronger limitation of dFe. At the same time, POC (and < 20  $\mu$ m *Chl a) concentrations did show an increase over time in the control (and T) treatment of bioassay W1.* Moreover, bioassay W2, with even lover starting concentrations of dFe, showed an increase in Chl a over time for the control. Given the lowest dMn concentrations in W1, it might be that dMn and not (only) dFe was limiting the production of reaction centres (Raven et al.; 19990), resulting in Chl a concentrations to not increase. Given the increased requirement for Mn under low Fe (Peers & Price; 2004), Fe addition may have relieved Mn limitation in the Fe addition treatments slightly, resulting in the observed increase of ChI a in those (F and TF) treatments."

Moreover, we will add the following on the Alderkamp et al. 2019 paper to line 675: "In addition to higher light levels, the lower initial dFe concentrations in the Ross Sea study (Alderkamp et al. 2019) compared to our study indicate a stronger Fe limitation and subsequently a stronger response to dFe addition."

-Line 680: The Fv/Fm results are minimally discussed, and do not provide much insight into the changes in the phytoplankton health together with both the temperature and iron changes and the confounding influence on the changing communities.

As suggested by Reviewer 2, we will refer to the  $F_v/F_m$  results more often throughout the Discussion, where they strengthen our discussion points (e.g. in lines 594 – 596 and line 658 of the original manuscript), rather than leaving them out. We will link Fv/Fm with sampling time in the productive season following line 661 (original manuscript). Moreover, we will refer to  $F_v/F_m$  values to show that bioassay A2 may not have been limited by dFe concentrations (based on a comment by Reviewer 2 on line 677). We will also change the order of the Discussion on  $F_v/F_m$  values to avoid switching between bioassays: "Indeed, given that the Weddell Sea bioassays were performed early in the productive season, these results imply more severe Fe limitation in the Weddell Sea whereas any Fe limitation in the Amundsen Sea likely only develops later in the season. Consistent with the lower dFe concentrations was the reduced in-situ  $F_v/F_m$  of the phytoplankton in W1 and W2, which stayed low for non-Fe addition treatments throughout the experiments, as it is a common indicator of Fe stress in the Southern Ocean (Greene et al., 1992; Mills et al., 2012; Olson et al., 2000; Jabre and Bertrand, 2020). In addition, the low dMn concentration may have contributed to the low  $F_v/F_m$  (Wu et al., 2019). The decrease in  $F_v/F_m$  in the F and TF treatments towards the end of the Weddell Sea bioassays seem to indicate that the added Fe had depleted again to limiting conditions or that Mn became (co-)limiting." Following line 671, we will add (about bioassay A2): "The high initial  $F_v/F_m$  values suggest that the phytoplankton may not have been limited by dFe (under these low light conditions) and would only require more dFe once light intensities increased again (Strzepek et al.; 2019, Vives et al.; 2022, Latour et al.; 2023). The small increase in  $F_v/F_m$  in the Fe addition treatments may suggest growth became dFe limited during the incubation (Fe-addition did show a significant effect on  $F_v/F_m$  at the last day of the incubations), despite the light conditions remaining low."

#### Conclusion:

I struggle to clearly see all the concluding points made from this study, particularly in the latter half of the conclusion. The conclusion partly reads like a literature review and does not sufficiently highlight some of the main conclusions. This undermines the value based on the breadth of the experiments and outcomes of this study. I would suggest focusing on synthesizing key findings and clearly articulating the study's contributions and implications in the conclusion.

We will move respective parts where fitting (Introduction and Discussion) or leave out. We will change the Conclusion section focusing only on our immediate results and on an outlook for future experiments. We will delete lines 733 – 735 and 766 – 773. Lines 754 – 759 will be moved to the Introduction. Lines 762 – 766 will be moved to the Discussion. The Conclusions now read (changed and/or added text is underlined): "Our study stands out in that it combined trace metal 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). Bioassay incubations were performed under trace metal clean conditions (for the entire duration) and with temperature remaining stable over the course of incubations (maximum fluctuation of temperature  $\pm$ 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. Our data also shows the importance of considering other regional and/or seasonal factors potentially limiting phytoplankton growth, such as e.g. light availability (limiting light conditions in bioassay A2) and dMn availability (potentially limiting in W1), when studying the effect of future climate on Southern Ocean phytoplankton. Additionally, our data indicates a trend of increased uptake of trace metals under dFe limitation, suggesting there are many adaptive strategies employed by phytoplankton in navigating nutrient scarcities under varying environmental conditions, with potential impact on the stoichiometry of global (micro-) nutrient distributions due to the central role of the Southern Ocean.

In general, the addition of dFe was the primary factor for observed stimulatory effects, with temperature enhancing the effect of dFe. Especially large diatoms benefitted from Fe addition, although several smaller-sized phytoplankton populations showed enhanced abundances upon Fe addition. <u>Climate change is predicted to lead to a shift towards smaller phytoplankton (Deppeler &</u> <u>Davidson, 2017; Krumhardt et al., 2022). Our study shows, however, that enhanced Fe input</u> <u>counteracts this warming-induced shift, assuming macronutrients will not become limiting.</u> 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 <u>and performing multiple bioassays over the productive season</u> 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). 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."

-Line 753: The reference to Brookes and Crowe (2019) appears in the conclusion with the statement that dual treatments may affect the responses. However, this referenced statement does not seem to appear among the discussion points or even as an inference. *We decided to take this part out of the manuscript, since it did not fit well after all.* 

-Lines 754-759: These lines read as introduction sentences, rather than providing a strong context for the conclusion from the study: i.e. enhanced Fe input in such regions may *partly* overturn the warming-induced shift, given that macronutrients will not become limited. Consider revising as this is not a compelling conclusion of the study. *We will change the Conclusions to be more concise (please see our response to your previous* 

-Line 764: 'only will the flow of organic carbon through the food web be affected,' *We will change the text accordingly.* 

-Lines 767-773: Again, I feel that these literature points can be better contextualized to your actual results.

We agree and changed the Conclusions (see reply to above comment).

Data availability: It seems that one needs to have an account with the NIOZ dataverse to access the data presented in the manuscript. Will this be publicly accessible later on? Data should already be available using the password and username provided by the editors. However, data will be publicly accessible once the manuscript is accepted.

Supplementary Information:

comment on this).

Generally, the panel sizes of all figures in the manuscript and supplementary could benefit from being slightly larger, so as to better see trends and the differences between the treatments or days. *We will adapt the figures accordingly.* 

Supplement Figure S1: This gives a nice overview of the physical setup. However, I struggled to fully comprehend and follow the details presented in the 'Bioassay set-up' in the supplementary text. *We will go through the text and adapt where necessary.* 

Supplement Figure S2: 3 Typos: '...the Amundsen Sea A1 (a, e, i), A2 (b, f, j) and the Weddell Sea...' 'The black dotted line represents the control (C) treatment, the red solid line the temperature (T) treatment, the blue solid line the iron (F) treatment, and the purple solid line the combined temperature and iron (TF) treatment.'

It is not very clear to see the 'black dotted line' from the panels without having to zoom in significantly. Are these supposed to be 'black solid line'? Perhaps you could change the scale/size of the y-axis to facilitate larger panels.

The purple line seems to blend too well with the red line. Consider using another contrasting colour like green or darker purple?

'Averages of triplicates with error bars represent the standard deviation'. *We will change the figures accordingly.* 

Supplement Figure S3: Again here, is the 'black dotted line' maybe meant to be 'black solid line'? Same comment regarding the purple and red solid lines blending. *We will change the figures accordingly.*  Supplement Figure S4: Panel 'd' is missing brackets '(d)'. Thank you for pointing this out, will change the figures accordingly.

'Average biovolume was calculated using total phytoplankton volume assuming spherical cells and dividing by total phytoplankton abundances.'

Thank you for noticing the typos (missing brackets etc.). We will change the text and legend where necessary and will also increase figure size and change the colour of the TF treatment (currently purple) to either a different colour or a different shade of purple.