Reviewers' comments:

Reviewer #1 (Remarks to the Author):

In "Biogeochemical metrics reveal distinctive but unusual iron cycling in the Southern Ocean eddies" Ellwood et al discuss iron measurements taken in a Southern Ocean eddy. They find Fe cycling characteristics in the eddy, a cyclone, that are distinct from surrounding waters. They conclude that the isolation of waters due to the eddy's dynamics allow for those distinct characteristics to occur, and more generally, that these finding will help to better understand Southern Ocean eddy chl/biomass anomalies.

The study presents -to my knowledge- a unique assessment of natural iron cycling within a Southern Ocean eddy, comparing it to measurements taken in surrounding waters. The study contributes to explaining mechanisms causing biogeochemical anomalies of Southern Ocean eddies. Thus, it presents a valuable addition to the field. The paper is well written. I have only few comments (see below), mainly concerning referencing of previous literature and why the eddy features characteristic Fe cycling. I recommend the paper for publication.

Minor comments:
*Title: How about a shorter the title, sth like "Distinct iron cycling in a Southern Ocean eddy"? Also, this is a case study about one eddy- that is, I suggest to stay with the singular in the title (see also comment below on how representative the eddy is for other cyclones).
*General: I understand that Fe cycling in the eddy is distinct compared to surrounding waters because the eddy is isolating waters; though, it is not entirely clear to me if the iron conditions of the eddy are typical for conditions south of the SAF where the eddy is originating from? That is, is "mere" advection of water, including its material properties, the main player in setting the distinct eddy iron characteristics (as suggested, e.g., in L70/71/76)? Or do the iron characteristics of the eddy further evolve during the ~1 month after it's detachment from the SAF (as suggested, e.g., in L96)? Also, you highlight the high Fe-to-carbon ratio and efficient recycling/short residence times of Fe in the eddy – can you comment on the larger-scale potential biogeochemical and/or ecological implications?
*L20 "productivity … low" and L28 "persistent productivity": Appears contracting at first glance. I suggest to rephrase to make clearer. E.g,. ", even though low, sustained productivity"?
*L34/35 "a crucial role in...": Please provide references for this statement (e.g., references you use later on, see References comment below).
*L37 "typically have closed circulation leading to biogeochemical properties": I am somewhat hesitant with "typically" as the majority of eddies does not appear to be efficient in trapping waters, see also, e.g., Wang et al, 2015; the cyclone observed here rather appears to represent an extreme case in that it features a rather high temperature anomaly (see also comment below); can you comment on this?
*L46 "supply of Fe... usually via": Do you mean to say that the supply usually is provided via iron-enriched deeper waters? If so I suggest sth like "supply of Fe usually is provided via upwelling and upward mixing of deeper iron enriched waters..."; also, lateral advection of iron by eddies may play a role, too, may it not, see e.g., Xiu et al, 2011, as an example from the north Pacific?
*L59 "about 2C lower": This appears to be a distinct/intense eddy; see "typical"/mean SST anomalies of eddies, e.g., in Haumann et al, 2012, of (well) below 1C; the large temperature anomaly suggests that the eddy rather is an extreme case, likely special also in terms of its biogeochemistry, and not so much an eddy representative for most eddies (see also comment above); I am happy to be convinced otherwise, though. If possible, could you comment on how many of such distinct eddies occur in the region versus the number of weaker eddies with less pronounced physical/biogeochemical anomalies (e.g,. based on satellite SLA and SST/Chl)?
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*Fig1: I suggest to add SSH or SLA contours, a typical proxy to identify eddies.

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*L35: In my comment above, I ask to provide references here, you could include, e.g. the references you use later, 7, 8, Sheen et al 2014 & Pollard et al, 2006
*L39, refs 1, 7, 8: Remove Frenger et al, 2015, Pollard et al, 2006, Sheen et al, 2014 (possibly include Sheen/Pollard above, see pervious comment) and rather use observational papers that highlight local biogeochemical anomalies of eddies e.g., Lehahn et al, 2011.
*L41, refs 8: Remove Sheen et al, 2014, possibly include Ansorge et al, 2010
*L48, ref 1: Include paper with the original idea, Flierl, 1981
*L54ff (paragraph): Here you introduce the observations of the eddy; please include references early on in this paragraph that discuss the same eddy und use the same observations, e.g., ref 13/23, Moreau et al, 2017 and Patel et al, 2019.
*L163-167: I suggest to add a sentence here, embedding these conclusions/hypotheses on light/iron limitation in eddies in previous works; do the statements/hypotheses (dis)agree with findings/hypotheses, e.g., of the observational works of Dawson et al, 2018, Frenger et al, 2018, and the modeling work of Song et al, 2018? Also, Moreau et al, 2017, already mention low iron concentrations in this eddy and discuss what (iron/light/grazing....) may limit productivity in the eddy. Maybe this is just about making overly clear what main points you would like to convey with your paper.

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References:
*Ansorge et al., 2010, Polar, Biology, https://link.springer.com/article/10.1007%2Fs00300-009-0752-9
*Chelton et al, 2011, Progr in Oceanography, https://doi.org/10.1016/j.pocean.2011.01.002
*Flierl, 1981, Geophysical & Astrophysical Fluid Dynamics, https://doi.org/10.1080/03091928108208773
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*Moreau et al, 2017, GBC, https://agupubs.onlinelibrary.wiley.com/doi/full/10.1002/2017GB005669
*Patel et al, 2019, JGR, https://agupubs.onlinelibrary.wiley.com/doi/full/10.1029/2018JC014655
*Song et al, 2018, GRL, https://agupubs.onlinelibrary.wiley.com/doi/full/10.1029/2017GL076246
*Wang et al, 2015, GRL, https://agupubs.onlinelibrary.wiley.com/doi/full/10.1002/2015GL064089
*Xiu et al, 2011, Iron flux induced by Haida eddies in the Gulf of Alaska, GRL, https://agupubs.onlinelibrary.wiley.com/doi/full/10.1029/2011GL047946

Reviewer #2 (Remarks to the Author):

This manuscript presents new data on Fe concentrations and Fe isotopes in the Southern Ocean, and interprets these data in the context of a 1D model of isotope cycling and other biogeochemical data.

The manuscript does not present enough information about methods to judge whether the underlying data are correct. This is particularly worrisome because the information which the authors do present hints that some data may be incorrect. I urge the authors to engage in significant additional methods testing and/or present the results of any significant methods testing which has already been performed. My three most significant concerns regarding methods are below:

1) Blank for Fe isotopes. The authors state a methods blank of 0.4 ng for Fe isotopes, yet they use 200 uL of AG-MP1 resin for purification. AG-MP1 resin has quite a high resin blank, and multiple publications suggest resin blanks for 200 uL resin would be roughly an order of magnitude higher (e.g. Dauphas et al. Anal Chem 2004, 1 mL resin, 20 ng Fe blank; Conway et al Anal Chim Acta, 2013, 20 uL resin 0.3 ng Fe blank; and others). The process used here for determining blanks was not clearly explained, aside from a sentence about running a 50 mL sample, and then calculating "based on a 2 L sample". Wouldn't it have been more straightforward just to report the blank obtained for the 50 mL sample, as this represents at least a minimum blank for a 2L sample? In any case, if the authors are really able to provide a 10-fold reduction in blank compared to all previous studies using the same anion exchange resin, this should be highlighted and clearly described.

Even if the reported blank of 0.4 ng is correct, more information needs to be provided. For the lowest concentration samples, the blank would constitute about 30% of the entire Fe in the samples. Was any sort of blank correction done? In order to do such a blank correction, the authors would need to know not only the concentration of the blank (and it's variability) but also it's isotopic composition (and it's variability). How was this determined?

2)Fe uptake rates. Little information was provided about how Fe uptake rates were measured except that they were determined using 55Fe. However, such experiments are notoriously difficult to perform. Specifically, it is quite common in such experiments that some of the "uptake" just represents surface adsorption or precipitation, rather than true biological internalization. This would invalidate the author's conclusion about the recycling timescale of Fe beeing ~ 1 day. Was the added Fe bound to any sort of ligand? Do the authors have any way of knowing whether the Fe was actually translocated across the cell membrane, or just precipitated on the cell surface?

3)Fe concentrations. Surprisingly for a manuscript focused so heavily on Fe concentrations, the authors appear not to have described the methods they used to measure Fe concentrations. Typical blanks for Fe concentration by Seafast are 150 pM. Blanks for Fe concentration analysis based on large-volume stable isotope samples may be smaller, but they would suffer from the same lack of clear description as described for δ56Fe in point 1. What sort of ICPMS instrument was used to measure Fe concentrations? What was the blank for this method? How was blank and blank variability determined?

Reviewer #3 (Remarks to the Author):

In this paper the authors present an analysis of the Fe chemistry of a Southern Ocean cold-core eddy. The analysis of Fe speciation and uptake rates is shown to infer that, while biomass and productivity in the eddy is low, the Fe uptake rate is high, suggesting rapid turnover of the Fe pool. The control of Fe availability on production in the S. Ocean has been extensively studied and this paper focusing on the role of mesoscale eddies adds to this story. The paper is well written and the data presented seems robust.

I felt however that the message of the paper was not well stated. The abstract suggests that a main finding is that fast Fe turnover sustains 'persistent production' even when Fe is low; however, the concluding remarks suggest that the main message is 'extreme Fe limitation is prevalent in summer and autumn'. This latter argument seems weaker than that suggested in the abstract (as we know Fe limits production in the S. Ocean). Can the authors relate the findings more to carbon and state/quantify the increase in production (albeit it low) as a result of fast recycling? This would strengthen the findings of the paper.
Minor comments:

A legend (similar to that on Figure 2) on figure 3 would help.

We thank the three reviewers for their constructive comments that have helped to improve the manuscript. Our responses to reviewer comments are highlighted in blue.

Reviewers' comments:

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Thank you for your positive and constructive comments.

Minor comments:
*Title: How about a shorter the title, sth like “Distinct iron cycling in a Southern Ocean eddy”? Also, this is a case study about one eddy- that is, I suggest to stay with the singular in the title (see also comment below on how representative the eddy is for other cyclones).

Amended. See line 1

*General: I understand that Fe cycling in the eddy is distinct compared to surrounding waters because the eddy is isolating waters; though, it is not entirely clear to me if the iron conditions of the eddy are typical for conditions south of the SAF where the eddy is originating from? That is, is “mere” advection of water, including its material properties, the main player in setting the distinct eddy iron characteristics (as suggested, e.g., in L70/71/76)? Or do the iron characteristics of the eddy further evolve during the ~1 month after it's detachment from the SAF (as suggested, e.g., in L96)? Also, you highlight the high Fe-to-carbon ratio and efficient recycling/short residence times of Fe in the eddy – can you comment on the larger-scale potential biogeochemical and/or ecological implications?

The dFe characteristics of the eddy do appear to have evolved as the eddy has aged. We have explored with the macronutrient concentrations which are slightly lower than for the waters where eddy developed. This is mentioned on lines 79 to 86.

We have included a sentence in the at the main text highlighting that eddy conditions would favor small cells and thus reduce carbon export. See lines 179 to 182.

*L20 “productivity … low” and L28 “persistent productivity”: Appears contracting at first glance. I suggest to rephrase to make clearer. E.g,. “, even though low, sustained productivity”?

Rephrased to “with cells upregulating iron uptake and using recycling processes to sustain themselves” see line28

*L34/35 “a crucial role in...”: Please provide references for this statement (e.g., references you use later on, see References comment below).

Two references have been added; Patel et al. (2019) and Patel et al. (2019). See line 35

*L37 “typically have closed circulation leading to biogeochemical properties”: I am somewhat hesitant with “typically” as the majority of eddies does not appear to be efficient in trapping waters, see also, e.g., Wang et al, 2015; the cyclone observed here rather appears to represent an extreme case in that it features a rather high temperature anomaly (see also comment below); can you comment on this?

The system that we studied is a akin to what Frenger et al. (2018) classified as a trapping or monopole eddy. We have rephrased lines section to mention this in the text. See line 37

*L46 “supply of Fe... usually via”: Do you mean to say that the supply usually is provided via iron-enriched deeper waters? If so I suggest sth like “supply of Fe usually is provided via upwelling and upward mixing of deeper iron enriched waters...”; also, lateral advection of iron by eddies may play a role, too, may it not, see e.g., Xiu et al, 2011, as an example from the north Pacific?

Eddies may play a role in nutrient and iron vertical and lateral supply depending on where they form. The warm core Haida eddy referred to in the Xiu et al. (2011) study transported warmer, fresher, and nutrient- and iron-enriched water offshore from the British Columbian coast. For our cold core eddy we observed elevated nutrient levels relative to the surrounding waters, but these levels are lower than those in the waters where the eddy likely formed. The consumption of nutrients within the eddy during its transit may well explain why dissolved iron is lower than in the surrounding waters. We have mentioned nutrient consumption on lines 79 to 86

*L59 “about 2C lower”: This appears to be a distinct/intense eddy; see “typical”/mean SST anomalies of eddies, e.g., in Haumann et al, 2012, of (well) below 1C; the large temperature anomaly suggests that the eddy rather is an extreme case, likely special also in terms of its biogeochemistry, and not so much an eddy representative for most eddies (see also comment above); I am happy to be convinced otherwise, though. If possible, could you comment on how many of such distinct eddies

occur in the region versus the number of weaker eddies with less pronounced physical/biogeochemical anomalies (e.g,. based on satellite SLA and SST/Chl)?

We have amended to the text to account for this point. Our was typical in size and life span but was more intense in terms of rotation speed and temperature gradients with respect surrounding waters. See lines 58-64.

*L77 “unique”: Unique compared to what, e.g., “unique in this region” or so. Or delete the second part of the sentence – is it necessary here?

Corrected.

*Fig1: I suggest to add SSH or SLA contours, a typical proxy to identify eddies.

We have included an extra panel to Figure 1 showing the sea level anomaly (SLA) for the region. The panel highlights that the eddy is characterised by lower SLA compared to surrounding waters. For decided to leave in the temperature contours for panels 1b and 1c as the nice define the edge of the eddy.

Referencing, a few suggestions:
*L34, ref 2: Include the observational paper Chelton et al, 2011, instead of the modeling work by Thomson et al 2010 (which focuses on fronts rather than eddies)?

Done.

*L35: In my comment above, I ask to provide references here, you could include, e.g. the references you use later, 7, 8, Sheen et al 2014 & Pollard et al, 2006

Amended so this now include (Moreau et al., 2017; Patel et al., 2019; Pollard et al., 2006; Sheen et al., 2014). Line 35

*L39, refs 1, 7, 8: Remove Frenger et al, 2015, Pollard et al, 2006, Sheen et al, 2014 (possibly include Sheen/Pollard above, see pervious comment) and rather use observational papers that highlight local biogeochemical anomalies of eddies e.g., Lehahn et al, 2011.

Done. Line 40

*L41, refs 8: Remove Sheen et al, 2014, possibly include Ansorge et al, 2010

Amended. Line 41

*L48, ref 1: Include paper with the original idea, Flierl, 1981

Done. line 48

*L54ff (paragraph): Here you introduce the observations of the eddy; please include references early on in this paragraph that discuss the same eddy und use the same observations, e.g., ref 13/23, Moreau et al, 2017 and Patel et al, 2019.

Amended. Line 55

*L163-167: I suggest to add a sentence here, embedding these conclusions/hypotheses on light/iron limitation in eddies in previous works; do the statements/hypotheses (dis)agree with findings/hypotheses, e.g., of the observational works of Dawson et al, 2018, Frenger et al, 2018, and the modeling work of Song et al, 2018? Also, Moreau et al, 2017, already mention low iron concentrations in this eddy and discuss what (iron/light/grazing....) may limit productivity in the eddy.

Done see lines 174 to 177

Maybe this is just about making overly clear what main points you would like to convey with your paper.

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References:

*Ansorge et al., 2010, Polar, Biology, https://link.springer.com/article/10.1007%2Fs00300-009-0752-9
*Chelton et al, 2011, Progr in Oceanography, https://doi.org/10.1016/j.pocean.2011.01.002
*Flierl, 1981, Geophysical & Astrophysical Fluid Dynamics, https://doi.org/10.1080/03091928108208773
*Frenger et al, 2018, Biogeosciences, https://www.biogeosciences.net/15/4781/2018/
*Hausmann et al, 2012, The observed signature of mesoscale eddies in sea surface temperature and the associated heat transport, DSR, https://linkinghub.elsevier.com/retrieve/pii/S0967063712001720
*Kahru et al., 2007, GRL, https://agupubs.onlinelibrary.wiley.com/doi/full/10.1029/2007GL030430
*Lehahn et al, 2011, Long range transport of a quasi isolated chlorophyll patch by an Agulhas ring, GRL, https://agupubs.onlinelibrary.wiley.com/doi/full/10.1029/2011GL048588
*Moreau et al, 2017, GBC, https://agupubs.onlinelibrary.wiley.com/doi/full/10.1002/2017GB005669
*Patel et al, 2019, JGR, https://agupubs.onlinelibrary.wiley.com/doi/full/10.1029/2018JC014655
*Song et al, 2018, GRL, https://agupubs.onlinelibrary.wiley.com/doi/full/10.1029/2017GL076246
*Wang et al, 2015, GRL, https://agupubs.onlinelibrary.wiley.com/doi/full/10.1002/2015GL064089
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This manuscript presents new data on Fe concentrations and Fe isotopes in the Southern Ocean, and interprets these data in the context of a 1D model of isotope cycling and other biogeochemical data.

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underlying data are correct. This is particularly worrisome because the information which the authors do present hints that some data may be incorrect. I urge the authors to engage in significant additional methods testing and/or present the results of any significant methods testing which has already been performed. My three most significant concerns regarding methods are below:

Thank you for your constructive comments. We have addressed you concerns below

1) Blank for Fe isotopes. The authors state a methods blank of 0.4 ng for Fe isotopes, yet they use 200 uL of AG-MP1 resin for purification. AG-MP1 resin has quite a high resin blank, and multiple publications suggest resin blanks for 200 uL resin would be roughly an order of magnitude higher (e.g. Dauphas et al. Anal Chem 2004, 1 mL resin, 20 ng Fe blank; Conway et al Anal Chim Acta, 2013, 20 uL resin 0.3 ng Fe blank; and others). The process used here for determining blanks was not clearly explained, aside from a sentence about running a 50 mL sample, and then calculating "based on a 2 L sample". Wouldn't it have been more straightforward just to report the blank obtained for the 50 mL sample, as this represents at least a minimum blank for a 2L sample? In any case, if the authors are really able to provide a 10-fold reduction in blank compared to all previous studies using the same anion exchange resin, this should be highlighted and clearly described.

We have increased the detail within the relevant Section, and have spelled out the blanks we obtained for the anion-exchange separation component of the method, and for the full iron extraction and separation process. For these we get blanks of 0.39 ± 0.34 ng (n = 4) and 0.40 ± 0.32 ng (n = 5), respectively. We have also detailed how we precleaned our AG-MP1 resin and how the columns were stored between use. At face value, most of the blank can be ascribed to the anion exchange process. See lines 227 to 306

With respect to the earlier work of Dauphas et al. (2004), their blank results were for the AG1-X8 and AG50W-X4 resins, not the AG-MP1 resin.

Even if the reported blank of 0.4 ng is correct, more information needs to be provided. For the lowest concentration samples, the blank would constitute about 30% of the entire Fe in the samples. Was any sort of blank correction done? In order to do such a blank correction, the authors would need to know not only the concentration of the blank (and it's variability) but also it's isotopic composition (and it's variability). How was this determined?

We have included more details about how we dealt with blank contribution within samples. Because we were not able to determine the iron isotope composition of the blank we chose not to correct the results. For all our results, a blank correction of the isotope data would have resulted in values that are statistically indistinguishable from the results presented. For example, for the 70m sample (2 ng of Fe) from the cold core eddy, we obtained a dissolved Fe isotope value of 1.10 ± 0.43 ‰ (2.SE). Correcting this 70m value with a 20% blank contribution (and assuming a lithogenic isotope value for the blank of 0.1‰) produces a result of 1.35 ± 0.43 ‰. The difference between this and the measured value is not statistically significant. Likewise, if we take the dissolved iron

isotope value for the 300 m depth sample (total of 45 ng Fe) from the cold core eddy, we obtain a blank corrected isotope value of 0.64 ± 0.05 ‰ which is indistinguishable from its measured isotope value of 0.64 ± 0.05 ‰.

Because we chose not to correct our iron isotope results we also chose not correct our concentration results. For the majority of the samples, this had no significant bearing on the final results. For a few this correction will be significant and thus they represent upper concentration bound.

See lines 301-306 and 326-327

2)Fe uptake rates. Little information was provided about how Fe uptake rates were measured except that they were determined using 55Fe. However, such experiments are notoriously difficult to perform. Specifically, it is quite common in such experiments that some of the "uptake" just represents surface adsorption or precipitation, rather than true biological internalization. This would invalidate the author's conclusion about the recycling timescale of Fe beeing ~ 1 day. Was the added Fe bound to any sort of ligand? Do the authors have any way of knowing whether the Fe was actually translocated across the cell membrane, or just precipitated on the cell surface?

Earlier field studies used $^{55}Fe$ concentrations one to two orders of magnitude higher (i.e., 2 – 20 nmol $L^{-1}$) than we used in this study (Maldonado et al. 2005, Strzepek et al. 2005, McKay et al. 2005, Hopkinson et al. 2012). In those cases, the addition of Fe bound to EDTA (usually 10 µM) was warranted. However, we added a much lower concentration of $^{55}Fe$ in our experiments: 0.2 nmol $L^{-1}$ of $^{55}Fe$ was added to each sample bottle as an acidified $^{55}FeCl_3$ solution (0.1 M Quartz-distilled HCl). No EDTA was used to complex the $^{55}Fe$ as the concentration of $^{55}Fe$ added is below the empirically observed threshold for precipitation of Fe (oxy)hydroxides (~ 0.7 nmol $L^{-1}$; Sunda and Huntsman 1995).

*Surface adsorption or precipitation, rather than true biological internalization:* The Ti(III) EDTA-citrate reagent that we used in our experiments was developed 30 years ago to remove extracellularly bound Fe from phytoplankton cells. The use of the Ti(III) EDTA-citrate reagent has been shown to be exceptionally successful in removing both freshly formed and aged particulate abiotic ferric oxyhydroxides (> 99% Fe removal; Hudson and Morel 1989, Tang and Morel 2006, Hassler and Schoemann 2009). The results obtained in the original paper describing the method (Hudson and Morel 1989) have been verified several times for both lab and field samples (Twining et al. 2004, Tang and Morel 2006, Hassler and Schoemann 2009). The Ti(III) reagent has shown to be an efficient washing agent as it relies on both redox potential and strong binding affinity for Fe(III) to first reduce $^{55}Fe$ oxyhydroxides that may precipitate on the cell surface, and then subsequently chelates the $^{55}Fe$ liberated

into the dissolved phase. The extracellular $^{55}Fe$ is then removed from the filters with multiple seawater rinses.

Combining data from all filter fractions and locations for the present study, Fe uptake rates and Fe:C uptake ratios were 2.65 ± 0.14 (±SE, *n* = 54) times higher, and C uptake rates 1.07 ± 0.03 times higher in unwashed samples compared to Ti(III) reagent-washed samples, indicating that: 1) intracellular Fe accounted for ~38% of total Fe 'uptake'; and 2) the Ti(III) EDTA – citrate reagent did not damage cells appreciably, as indicated by the good agreement in the C uptake rates between washed and unwashed filters.

We note also that the authors have a proven, >15 year, track record conducting these dual-label radiotracer experiments in the field, and have published extensively on how $^{55}Fe$ concentrations, $^{55}Fe$ chelation, and the use of the Ti reagent influence Fe:C uptake rates (e.g. Maldonado et al. 2005, Strzepek et al. 2005, McKay et al. 2005).

When a low concentration of $^{55}Fe$ is used (e.g. 0.2 nmol $L^{-1}$) and the cells are then washed with the Ti(III) reagent, Fe quotas measured with the radiotracer method are in good agreement with those obtained from single-cell measures of Fe content using synchrotron x-ray fluorescence (SXRF; Twining et al. 2004, Strzepek et al. 2005).

See lines 223 to 251

3)Fe concentrations. Surprisingly for a manuscript focused so heavily on Fe concentrations, the authors appear not to have described the methods they used to measure Fe concentrations. Typical blanks for Fe concentration by Seafast are 150 pM. Blanks for Fe concentration analysis based on large-volume stable isotope samples may be smaller, but they would suffer from the same lack of clear description as described for δ56Fe in point 1. What sort of ICPMS instrument was used to measure Fe concentrations? What was the blank for this method? How was blank and blank variability determined?

In the Methods section, we now present the blanks in two forms: the blank associated with anion-exchange separation component of the method, and for the full iron extraction and separation from each sample. To determine, the dissolved iron concentration we made use of the amount of double spike used to determine the iron isotope composition of each sample. Specifically, we state that "Dissolved iron concentration for each sample is calculated from sample volume and the amount double spike added to the sample. This calculation is based on isotope dilution using the known proportion of 58Fe in the 57Fe–58Fe double spike. Note that the dFe concentrations presented here were not blank corrected, thus, they represent an upper concentration bound." The instrument used to make iron isotope measurements was a ThermoScientific NeptunePlus which is mentioned in the methods section.

See lines 324 to 327

Reviewer #3 (Remarks to the Author):

In this paper the authors present an analysis of the Fe chemistry of a Southern Ocean cold-core eddy. The analysis of Fe speciation and uptake rates is shown to infer that, while biomass and productivity in the eddy is low, the Fe uptake rate is high, suggesting rapid turnover of the Fe pool. The control of Fe availability on production in the S. Ocean has been extensively studied and this paper focusing on the role of mesoscale eddies adds to this story. The paper is well written and the data presented seems robust.

Thank you for your positive and constructive comments.

I felt however that the message of the paper was not well stated. The abstract suggests that a main finding is that fast Fe turnover sustains 'persistent production' even when Fe is low; however, the concluding remarks suggest that the main message is 'extreme Fe limitation is prevalent in summer and autumn'. This latter argument seems weaker than that suggested in the abstract (as we know Fe limits production in the S. Ocean). Can the authors relate the findings more to carbon and state/quantify the increase in production (albeit it low) as a result of fast recycling? This would strengthen the findings of the paper.

We have reworded the last sentence in question in the Abstract, and also the last paragraph of the main text. See lines 26 to 30 and 179 to 184

Minor comments:

A legend (similar to that on Figure 2) on figure 3 would help.

Done. – we have station abbreviations on these panels

**References**

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# Distinct iron cycling in a Southern Ocean eddy

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**Abstract**

Mesoscale eddies are ubiquitous in the ocean, controlling ocean-atmosphere heat and gas exchange. However, their physical influence on phytoplankton production in the iron-limited Southern Ocean remains unknown. Here, we probed Fe cycling in a subantarctic cold-core eddy during the austral autumn. In-eddy, surface dissolved Fe concentrations and phytoplankton productivity and biomass were exceedingly low relative of external waters. In contrast, in-eddy phytoplankton Fe-to-carbon uptake ratios were elevated 2-6 fold, indicating upregulated intracellular Fe acquisition, thus reducing the residence time of Fe in the dissolved pool to ~1 day. Dissolved Fe isotope values were isotopically heavy in the euphotic zone, indicative of extensive trafficking of Fe by cells within the eddy. Below the euphotic zone, Fe isotope values were lighter and coincident with peaks in recycled nutrients and cell abundance, again indicative of enhanced microbially-mediated Fe recycling. Our measurements show that the isolated nature of eddies can produce distinctly different Fe biogeochemistry compared to surrounding waters with cells upregulating iron uptake and using recycling processes to sustain themselves. Recognising these eddy properties is essential to understanding how they contribute to the biophysicochemical structure of the Southern Ocean.

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**Main text**

Mesoscale eddies are ubiquitous in the ocean[1,2] and play a crucial role in the transfer of heat, carbon and nutrients between the deeper ocean, surface waters and the atmosphere[3-6]. Cold-core eddies in the Southern Ocean are defined by strong clockwise rotation, cooler temperatures and negative sea-surface height anomalies[7,8]. These eddies can have closed circulation thus 'trapping' [ref 9] the biogeochemical properties of these water such as nutrients, chlorophyll and particle concentrations differ compared to external waters[2,9-11]. They can transport these biogeochemical properties vast distances[12] thus they are important from an oceanographic point of view, especially if they cross water mass boundaries such as the Polar Front or the Subantarctic Front[7,11,13].

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The concentration of dissolved Fe (dFe) in remote Southern Ocean surface waters, away from continental and island input sources, is typically sub-nanomolar (60-200 pmol $kg^{-1}$) [ref 14,15]. The lower limit for this dFe range is thought to be controlled by organic complexation and atmospheric supply. In the Southern Ocean, atmospheric inputs are very low and the supply of Fe usually is provided via upwelling and upward mixing of deeper iron-enriched waters[14,16]. However, eddies can become

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'structurally closed' post-development, so their ability to entrain and detrain dFe, nutrients, phytoplankton and zooplankton can be restricted[1,17], thus making them a static 'mesocosm-like' environment. Structural breakdown of the eddy and the export of sinking organic matter are the principal biogeochemical loss terms. Our study focused on one such eddy to understand the biogeochemical cycling of Fe in this isolated structure. We contextualise our in-eddy Fe speciation (dissolved and particulate) and isotope measurements with primary productivity and biological observations and contrast them to external sites within the Subantarctic zone.

Between 30 March and 4 April 2016, we sampled a cyclonic cold core eddy that had spawned from the northern jet of the Subantarctic Front in mid-February 2016[ref 3,4]. Satellite-derived sea surface temperature imagery, vertical profiles of temperature, salinity and nutrients, and undulating sensor measurements revealed that the eddy was biogeochemically distinct from the waters surrounding it at two reference stations (SAZ and SOTS; Figures 1, S1, S2 and Video S1). This eddy was typical with respect to its size (diameter) and life-span with that of other eddies in the region [3], however it was more intense in terms of rotational speed and amplitude. Sea surface temperatures in the eddy were about 2°C lower than surrounding waters, again more intense than basin-averaged anomalies of about 0.5°C [ref 1]. Chlorophyll concentrations were approximately 1.5 times lower within the eddy, consistent with the analysis of 'trapping' eddies[9,11]. Large differences in fluorescence and transmissivity with depth inside the eddy highlight low biological production at the time of sampling (Figure S1), which we confirmed with *in situ* biological measurements. In-eddy pico-plankton and nano-plankton cell numbers and primary productivity rates were reduced compared to external stations (Figures 2, S3 and S4). In-eddy primary productivity declined from 0.162 mmol C $m^{-3}$ $d^{-1}$ at 80% incident irradiance (15 m) to 0.011 mmol C $m^{-3}$ $d^{-1}$ at 1% incident irradiance (100 m). Outside of the eddy, primary productivity was higher, with values of 0.30 mmol C $m^{-3}$ $d^{-1}$ and 0.38 mmolC $m^{-3}$ $d^{-1}$ at 80% incident irradiance (15 m) at the SAZ and SOTS sites, respectively. This is consistent with productivity (~0.3 mmol C $m^{-3}$ $d^{-1}$) previously determined for the region[18], and about three times higher than in-eddy rates. These observations are all consistent with the eddy originating south of the northern extension of the Subantarctic Front where temperatures and chlorophyll concentrations were lower (Figure 1 and Video S1 )[4].

In the Southern Ocean, Fe limits phytoplankton production seasonally[16,18]. Our in-eddy dFe concentrations are amongst the lowest ever measured for the Southern Ocean, with values between 18 and 33 pmol $kg^{-1}$ for the upper 200 m (Figures 3 and S2). Because the eddy developed in the northern jet of the Subantarctic Front, the Fe status of the eddy likely reflects the initial biogeochemistry of waters further south but modified on its northward transit. Indeed, nitrate and

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phosphate concentrations at 53°52'S; 147°16'E for waters near where the eddy formed were 2.3 µmol $L^{-1}$ and 0.1 µmol $L^{-1}$ higher, respectively than in-eddy levels suggesting biogeochemical modification of surface water properties as the eddy evolved. Interestingly, silicate levels were similar between the two sites suggesting that changes in nitrate and phosphate drawdown are likely facilitated by non-siliceous phytoplankton. Previous nearby summertime measurements for dFe from near where the eddy spawned, at and south of the Subantarctic Front, ranged between 70 and 210 pmol $kg^{-1}$, thus these low in-eddy dFe values appear to result from in-eddy processes (Table 1). Outside the eddy, dFe concentrations were 35 to 68 pmol $kg^{-1}$ and 57 to 210 pmol $kg^{-1}$ for the SAZ and SOTS stations, respectively (Figure 1), consistent with measurements for this region and the Southern Ocean generally (Table 1)[15]. Like dFe, in-eddy particulate Fe (pFe) concentrations were extremely low with values between 13 and 29 pmol $kg^{-1}$ for the upper 200 m (Figure 3). These low pFe concentrations reflect the low input of lithogenic material (dust) into the Southern Ocean (see supporting text)[19]. This observation is supported by the fact that between 51 and 69 % of the pFe pool in the upper 250 m of the eddy is considered biogenic (Figure S5).

Our results raise the question: How can dFe be consumed to such low concentrations by the in-eddy phytoplankton community, compared to external waters? We explored this question by measuring the rate of Fe uptake by cells inside and outside of the eddy. In-eddy Fe uptake rates were higher at 15 m (80% incident irradiance) compared to rates outside the eddy at the two reference sites (Figure 2 and S2). With depth, in-eddy Fe uptake rates decreased, but they were always higher than rates measured for the SAZ site (Figure 2). In-eddy carbon-normalised Fe uptake rates (expressed as a Fe:C ratio) for the resident phytoplankton community were approximately 4-fold higher compared to the two references sites and historical measurements for the region (Table 1). This is a surprising result considering the low in-eddy biomass at the time of occupation (Figures S1 and S3). A higher Fe:C uptake ratio for in-eddy phytoplankton is consistent with upregulation of cellular Fe acquisition machinery, to help acquire dFe. Indeed Fe-limited phytoplankton have been shown to transport Fe into the cell at a faster rate than Fe-replete cells[20]. The structurally isolated nature of the eddy with respect to deep and surrounding waters (Figure S1) also reduced dFe replenishment from below and laterally. The diffusional supply of dFe into the euphotic zone (0-85 m) is estimated to be 0.25 nmol $m^{-2}$ $d^{-1}$ based on the dFe gradient between 200 and 70 m and an eddy diffusion coefficient of $K$ = 3 x$10^{-5}$ $m^2$ $s^{-1}$ (ref [16,21]). This diffusional dFe supply rate is approximately 7 x$10^3$ times lower than the Fe utilisation rate of 1.88 µmol $m^{-2}$ $d^{-1}$ across the euphotic zone, thus highlighting the extreme nature of this cold core eddy with respect to Fe supply. It is possible that $K$ values in this region might be as high as $10^{-4}$ $m^2$ $s^{-1}$, but values higher than $10^{-3}$ $m^2$ $s^{-1}$ are never observed[22] and for the diffusive supply and biological demand to match would require $K$ = 0.2 $m^2$ $s^{-1}$. Using the utilisation rate and a dFe

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inventory of 1.87 μmol Fe $m^{-2}$ for the euphotic zone, we estimate a residence time of 1 day for dFe. This short residence time indicates that Fe is being heavily trafficked within the euphotic zone between the dissolved pool and the microbial community. The increased importance of iron recycling favours smaller phytoplankton cells, which is reflected in the cell abundances, the size-fractionated iron uptake and the Fe:C ratio datasets (Figures S3 and S4).

The elevated in-eddy Fe:C uptake ratios also raise the question as to how phytoplankton are enhancing dFe uptake. Enhanced dFe uptake can occur through a combination of processes[14], including increased production of Fe transporters on the surface of cells, a reduction in cell size, the production of Fe binding ligands, and the use of Fe(III) reductase proteins to enhance Fe(II) production and hence the acquisition of Fe from organic complexes. We used the isotopic composition of dFe and pFe to probe Fe uptake within the eddy.

The isotope composition of dFe ($\delta^{56}Fe_{diss}$) showed distinct variability with depth and between stations (Figure 3). In the euphotic zone (0-85m) of the cold core eddy, $\delta^{56}Fe_{diss}$ values are isotopically heavy at +1.19 ‰ and distinct ($p < 0.005$, T-test) to that of pFe ($\delta^{56}Fe_{part}$) and the $\delta^{56}Fe_{diss}$ composition for waters below the euphotic zone (0.00 ‰ at 150 m). The $\delta^{56}Fe_{diss}$ composition for surface waters at this site are also isotopically distinct ($p < 0.005$) to $\delta^{56}Fe_{diss}$ values measured at the SAZ and SOTS stations (Figure 3). At these stations, euphotic zone $\delta^{56}Fe_{diss}$ and $\delta^{56}Fe_{part}$ are isotopically similar to each other but significantly lower ($p < 0.005$) than in-eddy $\delta^{56}Fe_{diss}$ values (Figure 3).

The heavy in-eddy $\delta^{56}Fe_{diss}$ values for the euphotic zone are consistent with biological fractionation during dFe acquisition by phytoplankton. Modelling of the $\delta^{56}Fe_{diss}$ dataset using a closed system model produced isotope fractionation factors ($\varepsilon$) of -2.3 ‰ for samples collected in the euphotic zone (15 to 100 m; Figure 4). Interestingly, the in-eddy $\delta^{56}Fe_{part}$ values for the euphotic zone are not consistent with an instantaneous or an integrated isotope fractionation process associated with a closed system model. Generally, the expectation is that as dFe is consumed, the pFe pool should become isotopically heavier for both the instantaneous and the accumulated product. While $\delta^{56}Fe_{part}$ does appear to be subtly heavier with decreasing dFe concentration, it is not consistent with closed-system dependency for the biological reduction of Fe(III) to Fe(II) by cells (Figure 4). The physical cycling of $\delta^{56}Fe_{part}$ and $\delta^{56}Fe_{diss}$ offers a possible explanation for this discrepancy: $\delta^{56}Fe_{part}$ is distributed downward through the euphotic zone without general modification by sinking, whereas changes in the $\delta^{56}Fe_{diss}$ with depth (or dFe concentration) across the euphotic likely represent mixing across the euphotic zone.

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Using a generalised 1-D model, we simulated these two different mechanisms over the seasonal cycle and found that the $\delta^{56}Fe_{diss}$ signal could be adequately modelled using isotope fractionation associated with just dFe uptake alone ($\varepsilon$ = -1 ‰) or a combination of isotope fractionation processes, namely dFe uptake ($\varepsilon$ = -0.6 ‰), pFe regeneration ($\varepsilon$ = +0.15 ‰), dFe scavenging from solution ($\varepsilon$ = -0.3 ‰) and dFe complexation to natural organic ligands ($\varepsilon$ = +0.6 ‰) (Figure 4 and S9, see supplementary material for extended discussion). The overall value for $\varepsilon$ is considerably smaller than the expected value (between -2 and -3 ‰ [ref 23]) for biological reduction of Fe(III) to Fe(II) by cells, suggesting that dFe isotope fractionation is likely associated with the rapid trafficking of Fe between the dissolved pool and cells (Figure 4). The recycling of Fe between pools may also amplify the $\delta^{56}Fe_{diss}$ signal. The model also produces $\delta^{56}Fe_{part}$ values within the range measured in the euphotic zone, supporting the idea that mixing and particle sinking are the primary mechanisms responsible for distributing the $\delta^{56}Fe_{diss}$ and $\delta^{56}Fe_{part}$ signals across the euphotic zone.

Iron bioavailability is known to influence the oxidation rates of ammonia and nitrite[24], such that both accumulate if Fe is limiting. An in-eddy minimum in $\delta^{56}Fe_{diss}$ (Figure 4) coincided with peaks in heterotrophic bacterial cell numbers (Figure S3) and nitrite concentration at 150 m, and just below the peak in ammonium concentration (Figure S5). This minimum in $\delta^{56}Fe_{diss}$ and the maxima in nitrite, ammonia and heterotrophic bacterial cell numbers provides evidence for Fe control of the microbial recycling of organic matter in the mesopelagic. Note that no localised minimum in $\delta^{56}Fe_{diss}$ or peaks in heterotrophic bacterial cell abundance or ammonium and nitrite occurred below the euphotic zone at the SAZ or SOTS stations (Figure S3 and S5) indicating that Fe limitation of the microbial component of the mesopelagic community is restricted to the eddy station (Figure S5). The model reveals seasonality in dFe and pFe concentrations suggesting that these signals of particulate Fe sinking and remineralization are initiated in spring and persist through to autumn (Figure S8), thus it is likely that their influence on Southern Ocean biogeochemistry may be even greater than suggested by the autumn observations presented here.

Satellite analysis of thousands of cyclonic eddies from the Southern Ocean reveals that they are generally cooler than surrounding waters but have higher chlorophyll concentrations in winter and spring and lower chlorophyll concentrations in summer and autumn[11,25]. These eddies have shallower mixed layers, particularly in winter and spring[25,26]. The increase in production during winter and spring likely results from an alleviation of light limitation[27], which is a key variable limiting plankton growth after winter mixing has reset the dFe concentration from levels that can limit phytoplankton production[16]. In contrast, shallow mixed layers during summer result in cyclonic eddies having a lower dFe inventory and hence low phytoplankton production compared to

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surrounding waters[25]. Our study confirms that cyclonic eddies can have extremely low dFe inventories, thus requiring the resident plankton to upregulate iron uptake machinery and use recycling activities to sustain themselves. Such conditions favour small, non-siliceous cells, thus reducing export. At any time there are about 1200 eddies in the Southern Ocean, and approximately 50% of them are cyclonic[26], thus the extreme Fe limitation of Southern Ocean phytoplankton, as reported here, is likely to be the prevalent state during summer and autumn[25].

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## Methods

*Voyage*

The cold-core cyclonic eddy was studied between 28 March and 3 April 2016 (Figure 1). The eddy was about 190 km in diameter and was a stable feature that had formed approximately one month prior to sampling (Figure 1, Video S1). It was formed by detaching from waters 2 degrees south near the Subantarctic Front[3,4]. Post formation, the eddy moved slowly northward across the northern extension of the subantarctic front (SAF-N; Video S1). As it moved, the eddy completed approximately 7 full rotations during is 109 day lifetime[3] The biogeochemical properties of the cold-core cyclonic eddy were contrasted with two other sites in the subantarctic zone. One was located at 51.90°S, 148.51°E, designated a Subantarctic zone site (SAZ) and the other at 46.77°S, 142.03°E, the Southern Ocean Time Series (SOTS) site (Figure 1).

*CTD, nutrient sampling*

Conductivity, temperature, and depth (CTD) profile data and water samples for nutrients and biological parameters were collected with a winch-lowered package consisting of an SBE 911plus CTD, a Turner Designs fluorometer, and a 24-bottle SBE 32 Carousel water sampler. Salinities were calibrated to standard seawater (International Association for the Physical Sciences of the Ocean). Samples for phosphate, nitrate, nitrite, ammonia and silicic acid were collected and analysed at sea on unfiltered samples using a Seal AA3 segmented flow system following the procedures outlined by Armstrong, et al. [28] and Wood, et al. [29].

*Trace metal Sampling*

Seawater samples for trace metal and isotope determination were collected using Teflon-coated, externally-sprung, 12-litre Niskin bottles attached to an autonomous rosette equipped with a Conductivity Temperature Depth (CTD) unit (SeaBird 911 plus, USA). Upon retrieval, the Niskin bottles were transferred into a clean container laboratory fitted with HEPA-filtered air workstations. Seawater samples for dissolved trace metal analysis were filtered through acid-cleaned 0.2-µm capsule filters (Supor AcroPak 200, Pall) and acidified with distilled nitric acid to a final pH ≤ 1.8. The sampling protocols followed recommendations in the 'GEOTRACES Cookbook' (http://www.geotraces.org/science/intercalibration/222-sampling-and-sample-handling-protocols-for-geotraces-cruises).

Particulate trace metal samples were collected *in situ* onto acid-leached 0.2-µm Supor (142 mm diameter) filters (Pall, Australia) using six large-volume dual-head pumps (McLane Research

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Laboratories) deployed at various water depths. For most profiles, one pump depth was used as a blank check whereby only 4 L of water was pumped through the filter. This filter was then processed using the same procedure as the other samples.

*Primary Productivity and Fe uptake*

Net primary productivity and Fe uptake rates were determined for water column samples collected at six depths between 0 and 100 m [ref 30]. Water samples were collected pre-dawn from trace metal clean Niskin bottles deployed on a trace metal rosette. Sampling depths were determined from *in situ* irradiance depth profiles obtained during midday CTD casts the day prior to collection. Samples were dispensed into 300 mL acid-washed polycarbonate bottles and spiked with 16 µCi of Sodium $^{14}$C-bicarbonate ($NaH^{14}CO_3$; specific activity 1.85 GBq mmol$^{-1}$; PerkinElmer) and 0.2 nmol L$^{-1}$ of an acidified $^{55}$Fe solution ($^{55}FeCl_3$ in 0.1 M Ultrapure HCl; specific activity 30 MBq mmol$^{-1}$; PerkinElmer). Six samples (5 light and one dark bottle per irradiance) were incubated for 24 hours in a deck-board incubator under natural sunlight at 6 light intensities (from 80 to 1.0 % of incident irradiance). Light attenuation was adjusted by varying the layers of neutral density mesh and measured with a Biospherical Instruments QSL2101 Quantum Scalar PAR Sensor. The temperature of the incubator was controlled by a continuous supply of surface seawater.

Upon completion of the 24-hour incubation, 4 replicate samples were serially vacuum-filtered (<10 mm Hg) through 20 µm, 2.0 µm, and 0.2 µm porosity polycarbonate filters (47 mm diameter; Poretics) separated by 200 µm nylon mesh. Two size-fractionated samples were washed with Titanium(III) EDTA – citrate reagent for 5 min to dissolve Fe (oxy)hydroxides and remove ferric ions bound to the outer membrane surface[31], and rinsed three times with 15 mL of 0.2 µm-filtered seawater [32,33] and the other two size-fractionated samples were rinsed only with 0.2 µm-filtered seawater. In addition, two samples were filtered through 0.2 µm filters (a total community 'light' control, and a total community 'dark' control). Data for the dark-corrected, size-fractionated samples rinsed with the Ti(III) EDTA – citrate reagent are reported here (i.e., intracellular Fe:C uptake ratios).

The filters were transferred to 20 mL scintillation vials (Wheaton) and acidified with 100 µL of 1.0 M HCl to volatilize any remaining inorganic carbon[30]. Samples were counted on a liquid scintillation counter (PerkinElmer Tri-Carb 2910 TR) with a dual-label counting protocol after the addition of 10 mL of liquid scintillation cocktail (UltimaGold, PerkinElmer). Unfiltered water samples (1 mL) were used to quantify the concentrations of added $^{14}$C and $^{55}$Fe, and Fe:C uptake ratios for the size

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fractions were calculated from specific activities after accounting for both added and ambient dissolved Fe concentrations.

*Cell counts*

Flow cytometric analyses were performed following protocols outlined by Marie, et al. [34]. Samples for cell counts were preserved with glutaraldehyde and stored frozen at -80 °C to protect against cell lysis and the loss of autofluorescence. Prior to analysis, frozen samples were rapidly thawed in a water bath at 70 °C for 3 min and aliquots were taken for autotrophic and prokaryote cell counts. Sample aliquots were kept on ice in the dark and promptly analysed on a Becton Dickinson FACScan flow cytometer fitted with a 488 nm laser. Milli-Q water was used as sheath fluid for all analyses. Before and after each run, samples were weighed to determine the amount of sample analysed.

Autotrophic cell abundance samples were prepared by pipetting 1 mL of sample to a clean 5 mL polycarbonate tube, with 2 µL of PeakFlow Green 2.5 µL beads (Invitrogen) added as an internal fluorescence and size standard. Each sample was run for 5 min at a high flow rate of 40 µL $min^{-1}$. Autotrophic cell populations were separated into regions based on their chlorophyll autofluorescence in red (FL3) versus orange (FL2) bivariate scatter plots. *Synechococcus* sp cells were determined from their high FL2 and low FL3 fluorescence. Pico- (<2 µm) and nanophytoplankton (2-20 µm) communities were determined from their relative cell size in side scatter (SSC) versus FL3 fluorescence bivariate scatter plots.

Samples for prokaryote cell abundance were prepared by pipetting 1 mL of sample to a clean 5 mL polycarbonate tube. Samples with high prokaryote cell counts were diluted to 1:10 with 0.2 µm filtered seawater (FSW) to remove underestimation of cell concentration from coincidence (100 µL sample in 900 µL FSW). Cells were stained for 20 min with 5 µL of SYBR Green I (Invitrogen) at a final dilution of 1:10,000. An additional 2 µL of PeakFlow Green 2.5 µL beads (Invitrogen) were added to the sample as an internal fluorescence and size standard. Each sample was run at a low flow rate of ~12 µL $min^{-1}$ for 3 min and prokaryote cell abundance was determined from bivariate scatter plots of SSC versus green (FL1) fluorescence.

*Iron isotope analysis*

Particulate samples for trace element and $\delta^{56}Fe$ determination were thawed and processed using previous acid digestion protocols[35-37]. For dFe isotope determination, seawater samples (2L) were spiked with a $^{57}Fe$-$^{58}Fe$ double spike (1-3:1 spike: sample ratio)[38,39]. Samples were left overnight to equilibrate, after which they were buffered to a pH of 4.5 with a trace-metal clean ammonium

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acetate buffer and then passed over 0.5 mL columns packed with Nobias PA Chelate PA1L resin (Hitachi-Hitec, Japan) at a flow rate of 2 mL $min^{-1}$. Samples were rinsed with 4 mL of ammonium acetate buffer solution (1% w $w^{-1}$) followed by elution with 4 mL of 1 mol $L^{-1}$ nitric acid. Samples were evaporated to dryness and redissolved with 0.5 mL of 6 mol $L^{-1}$ hydrochloric acid containing $H_2O_2$. Samples were further purified using an anion exchange procedure similar to that described by Poitrasson and Freydier [40]. Precleaning of the AG-MP1 resin involved rinsing with methanol, multiple washes with 6 mol $L^{-1}$ HCl and 0.5 mol $L^{-1}$ $HNO_3$ before storage in dilute $HNO_3$. When required ~200 µL columns filled with the precleaned anion exchange resin AG-MP1 (Bio-Rad), conditioned by washing with 0.5 mol $L^{-1}$ HCl, 0.5 mol $L^{-1}$ HNO3, Milli-Q water and finally 6 mol $L^{-1}$HCl before use. Between use, columns were stored filled in 2% w $w^{-1}$ $HNO_3$. Columns were typically used 5-8 times before being refilled with new precleaned resin. After sampling loading, salts and other elements not of interest were eluted from the column by passing 3x 1 mL of 6 mol $L^{-1}$ hydrochloric acid. Iron was eluted with 3x 1 mL of 0.5 mol $L^{-1}$ hydrochloric acid and evaporated to dryness. Samples were redissolved in either 0.30 or 0.35 mL of 2% (w $w^{-1}$) nitric acid. The blank associated with the anion exchange separation was 0.39 ± 0.34 ng (n = 4). Procedural concentration blanks for the whole process were determined by passing small volumes (~50 mL) of an in-house seawater standard with a concentration 0.78 ± 0.08 nmol $kg^{-1}$ over the Nobias PA Chelate PA1L resin and then through the whole elemental and Fe isotope separation procedure. The dissolved iron concentration for this smaller seawater was then scaled to 2L thus allowing us to estimate the blank associated with the buffering of the sample, passing it over the Nobias PA Chelate PA1L resin and then over the anion exchange columns. The blank associated with this test was determined to be 0.40 ± 0.32 ng (n = 5). Note that we were not able to determine the isotope composition of the blank associated with the extraction and processing procedure, so the isotope values presented in have not been blank corrected. For dissolved samples, the total amount of Fe analysed ranged between 2 and 63 ng, thus the contribution of the blank to the lowest concentration samples could have been between as much 20 ± 17% of the lowest $\delta^{56}Fe_{diss}$ signal, i.e. for samples collected from the upper water column within the CCE.

Iron isotopes were determined using a Neptune Plus multi-collector ICPMS (ThermoScientific) with an APEX-IR introduction system (ESI, USA) and with X-type skimmer cones. Samples were measured in high-resolution mode with $^{54}Cr$ interference correction on $^{54}Fe$ and $^{58}Ni$ interference correction on $^{58}Fe$. Fe isotope ratios ($^{56}Fe/^{54}Fe$) ratios are reported in delta notation (‰) relative to the IRMM-014 Fe isotope reference material (IRMM, Brussels) using the double spike ($^{57}Fe$-$^{58}Fe$) correction technique[38,39] where:

**Deleted:** Poitrasson and Freydier [34]. This procedure involved loading samples onto ~200 µL columns filled with the precleaned anion exchange resin AG-MP1 (Bio-Rad). Salts and other elements not of interest were eluted by passing 3x 1 mL of 6 mol $L^{-1}$ hydrochloric acid. Fe

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$$\delta^{56}Fe = \left(\frac{^{56}Fe/^{54}Fe_{sample}}{^{56}Fe/^{54}Fe_{IRMM-014}} - 1\right) \text{x } 1000 \quad (1)$$

The overall instrumental error for dFe and pFe samples ranged between ± 0.04 ‰ and ± 0.64 ‰ (2σ). For low concentration samples, the instrumental error increased with decreasing Fe concentration and was associated with instrumental noise (Figure S6)[41]. Multiple large volume (3x 2L) extractions and analysis of an in-house seawater standard had a reproducibility of 0.76 ± 0.07 ‰ (mean ± 2x standard deviation). Multiple analysis of a particulate sample had a reproducibility of 0.15 ± 0.06 ‰ (mean ± 2x standard deviation). Analysis of geological samples NOD-A-1 and BCR-2 produced values of -0.43 ± 0.03 ‰ and 0.04 ± 0.07 ‰ (mean ± 2x standard deviation), respectively, which were consistent with literature values of -0.42 ± 0.07 [ref 42] for NOD-A-1 and 0.03 ± 0.06 [ref 42] for BCR-2

Dissolved iron concentration for each sample was calculated using sample weight and the amount double spike added to the sample. This calculation is based on isotope dilution using the known proportion of $^{58}$Fe in the $^{57}$Fe–$^{58}$Fe double spike[38,43]. Note that the dFe concentrations presented here were not blank corrected, thus, they represent an upper concentration bound.

*Isotope modelling*

The closed system equation for the isotopic evolution of dFe as it is consumed can be described as follows:

$$\delta^{56}Fe_{dissolved} = \delta^{56}Fe_{dissolved.100m} + \varepsilon \ln(f) \quad (2)$$

Where ε represents the isotope enrichment factor between the product (biologically utilised Fe) and the substrate (dFe) and *f* presents the fraction of dFe relative to the concentration of dFe at 100 m. The evolution of the instantaneous or integrated isotope fractionation processes can be modelled using the following expressions:

$$\delta^{56}Fe_{particulate} = \delta^{56}Fe_{dissolved} - \varepsilon \quad (3)$$

$$\delta^{56}Fe_{particulate} = \delta^{56}Fe_{dissolved.100m} + \frac{\varepsilon \ln(f)}{1-f} \quad (4)$$

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*1D biogeochemical modelling*

The potential processes that influence the distribution and isotope fractionation of dFe and pFe were explored using a 1D model (Figure S7). The rationale for using this 1D model is to explore the relative influence (and interplay) of processes such as phytoplankton utilisation of Fe, its complexation to natural organic ligands, its regeneration from sinking organic matter and the role of scavenging on distribution and expression of isotope profiles. The model is based on Schlosser, et al. [44] and includes one phytoplankton group and references key nutrients including nitrate, phosphate and Fe (Figures S7, S8 and S9). The model includes mixing, which supplies nutrients into the euphotic zone and the main loss process for nutrients and Fe from the euphotic zone (organic matter export). The Fe component in the model also includes complexation to natural organic ligands, scavenging and the atmospheric supply of Fe through the deposition and dissolution of dust (Support Information, Figure S7)). The model also does not include advection, which we justify for several reasons: i) vertical advection, i.e. upwelling, occurs in the Southern Ocean primarily south of the Polar Front and not in the SAZ and SAF regions examined here for the cold core eddy[45], ii) latitudinal advection supplies waters with similar properties from upstream in the Antarctic Circumpolar Current[46], and can thus be ignored; and iii) transport is dominated by northward Ekman transport, and while this does supply nutrients over the annual mean, in late summer surface concentrations between the SAF and PFZ are very uniform[47], so this term can also be neglected. The equations and values associated with each biogeochemical process are presented in the Supporting materials and in Tables S1 and S2.

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**Acknowledgments**

This research was financially supported under Australian Research Council's Discovery program (DP170102108; DP130100679) and ship time from Australia's Marine National Facility. We are grateful to the officers, crew, and research staff of the Marine National Facility *R.V. Investigator* for their help with sample collection and generation of hydrochemistry data. We are grateful to Stacy Deppeler for running the flow cytometry samples. We are grateful for the comments from three anonymous reviewers for their thoughtful comments that helped to improve the manuscript.

**Author contributions**

M.J.E. and P.W.B. conceived the study with input from P.G.S. and T.W.T. on appropriate oceanographic sampling sites. M.J.E., R.F.S. and M.F. collected and analysed samples. M.J.E. wrote the paper with assistance from all co-authors.

**Table**

**Table 1.** Intracellular iron:carbon (Fe:C) ratios, Fe uptake rates and dissolved Fe concentrations for upper Southern Ocean waters between longitudes 142°E and 172°E.

| Station/Study Latitude | Fe:C (µmol mol$^{-1}$) | Fe uptake rate (pmol L$^{-1}$ d$^{-1}$) | Dissolved Fe (pmol kg$^{-1}$) | Notes | Reference |
|---|---|---|---|---|---|
| Cold core eddy - 49.7°S | 224 ± 38 | 25 ± 5 | 23 ± 1 | 15-40 m - 80-20% irradiance | this study |
| SAZ - 51.4°S | 40 ± 8 | 12 ± 2 | 48 ± 17 | 15-40 m - 80-20% irradiance | this study |
| SOTS - 46.7°S | 61 ± 7 | 28 ± 6 | 60 ± 4 | 15-40 m - 80-20% irradiance | this study |
| SOIREE - 61°S | 3-7.5[#] | 3.07[#] | 80 ± 30[$] | Fe enrichment experiment | Bowie et al. [48] |
| FeCycle - 46°S | 5.5 - 19 | 290-360 | 51 ± 11 | Subantarctic experiment | McKay et al. [49] |
| SOFex - 56°S | 9-11 | not reported | 140 | Northern Patch | Twining et al. [50] |
| SAZ Project - 47°S | 52 | 60 | 70 | SOTS station | Boyd et al. [18] |
| SAZ Project - 54°S | 78 | 55 | 70 | Polar water station - equivalent to where the cyclonic eddy originated | Boyd et al. [18] |
| SAZ-Sense P1 - 46.3°S | 70 ± 44 | 110 ± 10 | 260 ± 40 | Equivalent to SOTS station | Bowie et al. [51] |
| SAZ-Sense P2 - 54°S | 60 ± 9 | 34 ± 5 | 210 ± 20 | Polar water station - equivalent to where the cyclonic eddy originated | Bowie et al. [51] |
| SAZ-Sense P3 - 45.5°S | 74 ± 47 | 77 ± 10 | 440 ± 70 | Site close to Subtropical convergence | Bowie et al. [51] |

[$] Background dissolved Fe concentration before Fe infusion. [#] Fe:C and Fe uptake rates are for days 1-3 after Fe infusion.

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505 **Figure captions**

506 **Figure 1.** Chlorophyll and sea surface temperature in the study area. **a** and **b**, Maps of chlorophyll *a*
507 concentration **c**. Sea Surface Temperature (SST) and **d.** Sea Level Anomaly (SLA) for Southern Ocean
508 waters south of Australia. The diamonds represent the Cold Core eddy station (CCE), the
509 Subantarctic zone station (SAZ) and the Southern Ocean Time Series station (SOTS) and the solid
510 black line represents the Triaxus tow (Figure S1). The chlorophyll *a* satellite data represents a
511 monthly average for March 2016. The SST data are for 3 April 2016 and SLA data are for 25 March
512 2016. In **b** and **c** the contours lines are SST. Data extracted from
513 https://coastwatch.pfeg.noaa.gov/erddap/griddap/.

514

515 **Figure 2.** Depth profiles of Fe and carbon uptake. Profiles of **a**, Fe and **b**, carbon uptake versus depth.
516 **c**, Profiles of the intracellular Fe:C uptake ratio for the CCE, SAZ and SOTS stations.

517

518 **Figure 3.** Depth profiles of Fe concentration and isotope composition. Upper ocean (0 to 600 m)
519 depth profiles of dFe and pFe concentration (**a, b, c**), and the isotope composition (**d, e, f**) for
520 samples collected at the **a**., **d.,** CCE, **b.**, **e.,** SAZ and **c.**, **f.,** SOTS stations.

521

522 **Figure 4.** Iron isotope fractionation model calculations. **a.** Dissolved $\delta^{56}$Fe values for samples
523 collected at the CCE, SAZ and SOTS stations versus Fe concentration. **b**. Model curves for closed
524 steady-state isotope fractionation (equations 2 to 4) of dFe for the CCE. The best fit ε value for the
525 cold core eddy dFe data is -2.3 ‰. One-D model profiles (blue lines) for dFe and pFe versus depth for
526 **c.** ε equal to -1.0 ‰ ($\alpha_{uptake}$ = 0.999) and **d.** ε equal to -2.3 ‰ ($\alpha_{uptake}$ = 0.9977) along with profiles of
527 dFe and pFe isotope composition versus depth for the CCE.

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Reviewers' comments:

Reviewer #1 (Remarks to the Author):

Thank you for addressing my comments. From my (non-iron but eddy-focused) perspective, I am fine with the publication of the manuscript as it is.

Grammar:
* L37ff: The sentence "These eddies can have closed circulation thus 'trapping' the biogeochemical properties of these water such as nutrients, chlorophyll and particle concentrations differ compared to external waters. " needs correction.

Reviewer #2 (Remarks to the Author):

I can't shake the feeling that the Fe isotopes data could possibly be incorrect. Seawater Fe isotopes are quite challenging to measure, and the authors are presenting d56Fe for some of the lowest-Fe (and thus most challenging) waters ever reported. Also, they present a blank for purification of Fe on AG-MP1 resin which is lower than ever reported previously, by a factor of something like 5-10 by my rough estimation, without any obvious reason why their blank should be so much lower than other studies. Also, their reported blank of 0.4 +/- 0.32 ng Fe suggests great variability in this value, and thus uncertainty in d56Fe.

The key "gold standard" experiment which would allay my analytical concerns would be to remove Fe from seawater, then dope back in a small amount of non-crustal Fe standard (e.g. 50 pM Fe which is either +2 permil or - 2 permil), then extract that new standard Fe and show that the correct d56Fe value is obtained. Successfully performing such an experiment would be very convincing, and indeed such experiments are often presented by labs which are developing new seawater metal isotope methods. However, I recognize that, depending on whether they are still set up to measure Fe isotopes routinely, doing such experiments could require a fair amount of work, and it seems unfair to hold up publication of a paper until a whole new round of methods-development can be undertaken.

Alternatively, perhaps the authors could more honestly discuss the incredible challenges of measuring Fe at such low concentrations, and acknowledge that they have not yet done the "gold standard" experiment of doping an isotope standard back into Fe-free seawater (as described above). They could also provide even more information about issues such as the variability in their blank. Then they could discuss the reasons why they still believe that their data are sound, and reasons why any expected small errors in their measurement should not undermine their basic conclusions.

I appreciate that the authors have responded in a detailed fashion to my earlier concerns about methods. They have made a convincing argument that their Fe radioisotope uptake experiments and Fe concentration data are valid. And if the reported blanks and other methods details are taken at face value, then their d56Fe data should also be correct. The interpretation of the data is reasonable and the conclusions are significant.

I support eventual publication of this manuscript, and I don't wish to ask for an insurmountable amount of additional effort. If the authors are able to perform the "gold standard" experiment, that would greatly strengthen this manuscript, and it would clearly establish them as a lab capable of measuring Fe isotopes even in low-Fe Southern Ocean waters, which in turn would open up exciting new areas of research. Alternatively, I hope that an extraordinarily thorough and honest discussion of the strengths, and possible weaknesses, of their d56Fe methods can be included, as a step towards the eventual goal of establishing unequivocally how Fe isotopes cycle in the Southern Ocean.

Best,

Seth John

Reviewer #3 (Remarks to the Author):

I am happy the authors have addressed my comments.

Dear Dr Frischkorn,

We are grateful to all three reviewers for their comments on our manuscript. Below is our response to points raised by the reviewres.

Reviewers' comments:

Reviewer #1 (Remarks to the Author):

Thank you for addressing my comments. From my (non-iron but eddy-focused) perspective, I am fine with the publication of the manuscript as it is.

Grammar:

* L37ff: The sentence "These eddies can have closed circulation thus ‘trapping’ the biogeochemical properties of these water such as nutrients, chlorophyll and particle concentrations differ compared to external waters. " needs correction.

Response: We have revised this sentence so it now reads ”These eddies can have closed circulation thus ‘trapping’ ref 9 the biogeochemical properties of these features such that nutrient, chlorophyll and particle concentrations can be distinct relative to those in the surrounding waters2,9-11”

Reviewer #2 (Remarks to the Author):

I can’t shake the feeling that the Fe isotopes data could possibly be incorrect. Seawater Fe isotopes are quite challenging to measure, and the authors are presenting d56Fe for some of the lowest-Fe (and thus most challenging) waters ever reported. Also, they present a blank for purification of Fe on AG-MP1 resin which is lower than ever reported previously, by a factor of something like 5-10 by my rough estimation, without any obvious reason why their blank should be so much lower than other studies. Also, their reported blank of 0.4 +/- 0.32 ng Fe suggests great variability in this value, and thus uncertainty in d56Fe.

Response: In 2019 I (Michael Ellwood) published a paper describing iron isotope transformations in Lake Cadagno Switzerland (Ellwood et al., 2019). In that study, the anion exchange resin AG-MP1 was also utilised to separate iron from other ions for a freshwater study for a Swiss mountain lake. This work was undertaken in the labs at ETH Zurich and utilised their chemicals and their resin. The overall dissolved iron procedural blank of that study was 0.6 ± 0.5 ng (Ellwood et al., 2019), which included evaporating samples to dryness and passing the samples over the AG-MP1 resin. The blank from that study is comparable to the blanks results obtained in the present study (0.40 ± 0.32 ng) where column separation of iron was undertaken at the ANU using a different batch on AG-MP1 resin to that used at ETH. Subsequent to the analysis of the 2016 samples (i.e., results featured in our manuscript), we have managed to obtain the same, and in some instances lower, blank values for the AG-MP1 resin for processing other iron isotope samples. Thus, in the present study we

present a blank that is comparable to that reported previously, and which has subsequently been repeatable (and surpassed) at the ANU.

The key “gold standard" experiment which would allay my analytical concerns would be to remove Fe from seawater, then dope back in a small amount of non-crustal Fe standard (e.g. 50 pM Fe which is either +2 permil or - 2 permil), then extract that new standard Fe and show that the correct d56Fe value is obtained. Successfully performing such an experiment would be very convincing, and indeed such experiments are often presented by labs which are developing new seawater metal isotope methods. However, I recognize that, depending on whether they are still set up to measure Fe isotopes routinely, doing such experiments could require a fair amount of work, and it seems unfair to hold up publication of a paper until a whole new round of methods-development can be undertaken.

Response: Prior to the analysis of the samples from the 2016 voyage , we did indeed test that the ANU method was producing high-quality results. This was done by undertaking an inter-calibration exercise (perhaps the “platinum standard” as each analysis is completely independent) with Tim Conway and Matthies Sieber at ETH. In this inter-calibration exercise, samples from the GEOTRACES crossover station at 32.5°S, 170°W for the GP13 were analysed by the ETH group. The ANU results were also compared to iron isotope results generated by the ETH for samples collected GP19 voyage at 32.5°S, 170°W crossover station. The results from the two groups showed that were no systematic differences between methods used by the two groups – see the figure below. The results from this inter-calibration exercise were also presented at the 2018 Ocean Sciences meeting (Conway et al., 2018), and Conway et al., are in the process of preparing a manuscript detailing the results from this inter-calibration exercise.

Prior to developing the double spike method at the ANU, the ANU group also participated in the GEOTRACES inter-calibration exercise where groups were able to analyse samples collected from the BATS site near Bermuda. The results produced by the ANU group using the standard-sample-standard bracketing technique were comparable to the other groups undertaking iron isotope analysis (see the table below). This work was published by Boyle et al. (2012).

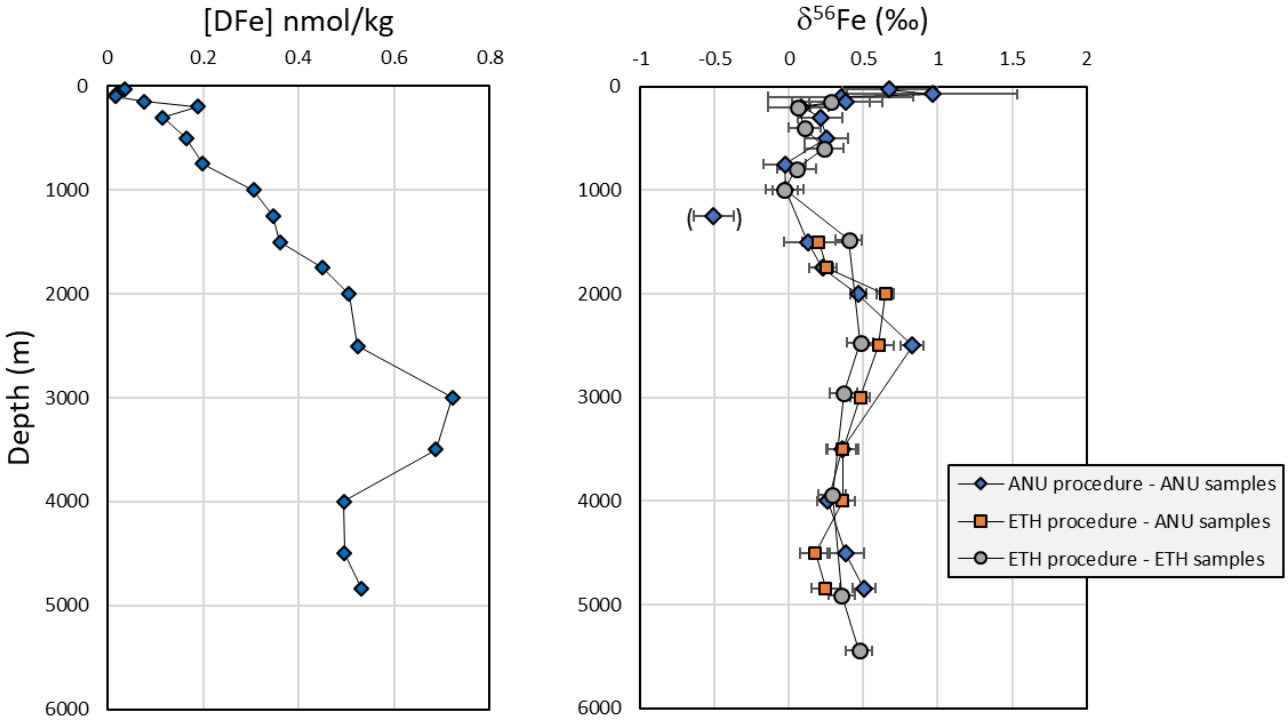
Overall, the ANU double spike method produces iron isotope results that are comparable to other field-leading interantional groups working in the space.

**Table 2.** Fe isotope data for GEOTRACES IC1 samples.

| Sample | Depth (m) | ANU $\delta^{56}Fe/^{54}Fe$ | WHOI $\delta^{56}Fe/^{54}Fe$ | Caltech $\delta^{56}Fe/^{54}Fe$ | LEGOS $\delta^{56}Fe/^{54}Fe$ | LEGOS [Fe] (nmol/kg) |
|---|---|---|---|---|---|---|
| GSI | 7 | 0.32±0.06(2se) | +0.24±0.10(2se n=3) | +0.32±0.06(2se n=2) | +0.41±0.04(2se n=3) | 0.42 |
| GDI | 2000 | 0.45±0.13(2se) | +0.42±0.11(2se n=3) | +0.55±0.03(2se n=5) | +0.52±0.07(2se n=2) | 0.84 |
| GPrI | 75 | | | +0.41±0.06(2se n=4) | | |
| GPrI | 125 | | | +0.30±0.06(2se n=5) | | |
| GPrI | 250 | | | +0.45±0.05(2se n=3) | | |
| GPrI | 500 | | | +0.34±0.05(2se n=2) | | |
| GPrI | 1000 | | | +0.35±0.05(2se n=2) | | |
| GPrI | 1500 | | | +0.35±0.05(2se n=2) | | |
| GPrI | 2500 | | | +0.71±0.05(2se n=2) | | |
| 4200m (individual sample) | 4200 | | | +0.35± 0.07(2se n=1) | | |

All $\delta^{56}Fe/^{54}Fe$ data are per mill relative to IRMM-014

Table 2 taken from Boyle et al. (2012) with the ANU results highlighted.




The above Figure presents the iron isotope results for samples collected on the GP13 and GP19 campaigns for a crossover station located at 32.5°S, 170°W in the South Pacific Ocean. The iron concentration results (left panel) are from the ANU group and have been published (Ellwood et al., 2018). The iron isotope results are for samples collected and analysed by the ANU group, samples the ANU group shared with the ETH group and samples collected on GP19 and analysed by the ETH group. One sample is highlighted as an outlier. The trends seen in the GP13 and GP19 profiles are consistent with each other and there is no systematic offset in the datasets, highlighting the quality of both the ANU and ETH methods.

Alternatively, perhaps the authors could more honestly discuss the incredible challenges of measuring Fe at such low concentrations, and acknowledge that they have not yet done the "gold standard" experiment of doping an isotope standard back into Fe-free seawater (as described above). They could also provide even more information about issues such as the variability in their blank. Then they could discuss the reasons why they still believe that their data are sound, and reasons why any expected small errors in their measurement should not undermine their basic conclusions.

Response: We have included the following sentences in the Methods section of the manuscript to highlight the quality of the iron isotope method. "The performance of the iron isotope method was also assessed through an inter-calibration exercise for samples from the GEOTRACES GP13 and GP19 campaigns at a crossover station located at 30°S; 170°W. The iron isotope results from the exercise were comparable – i.e., the trends seen in the GP13 and GP19 profiles are consistent with each other [43]."

I appreciate that the authors have responded in a detailed fashion to my earlier concerns about methods. They have made a convincing argument that their Fe radioisotope uptake experiments and Fe concentration data are valid. And if the reported blanks and other methods details are taken at face value, then their d56Fe data should also be correct. The interpretation of the data is reasonable and the conclusions are significant.

Response: Thank you

I support eventual publication of this manuscript, and I don't wish to ask for an insurmountable amount of additional effort. If the authors are able to perform the "gold standard" experiment, that would greatly strengthen this manuscript, and it would clearly establish them as a lab capable of measuring Fe isotopes even in low-Fe Southern Ocean waters, which in turn would open up exciting new areas of research. Alternatively, I hope that an extraordinarily thorough and honest discussion of the strengths, and possible weaknesses, of their d56Fe methods can be included, as a step towards the eventual goal of establishing unequivocally how Fe isotopes cycle in the Southern Ocean.

We have added the following sentence to the manuscript and point the reader to additional figure (S10) in the support materials highlighting the quality of the data. "The performance of the Fe isotope method was also assessed through an intercalibration exercise for samples from the GP13 and GP19 campiagns at a crossover station located at 30°S; 170°W. The iron isotope results from the exercise were comparable – i.e., the trends seen in the GP13 and GP19 profiles are consistent with each other (Figure S10) [43]"

Best,

Seth John

Reviewer #3 (Remarks to the Author):

I am happy the authors have addressed my comments.

Thank you

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Boyle, E.A., John, S., Abouchami, W., Adkins, J.F., Echegoyen-Sanz, Y., Ellwood, M., Flegal, A.R., Fornace, K., Gallon, C., Galer, S., Gault-Ringold, M., Lacan, F., Radic, A., Rehkamper, M., Rouxel, O., Sohrin, Y., Stirling, C., Thompson, C., Vance, D., Xue, Z. and Zhao, Y., 2012. GEOTRACES IC1 (BATS) contamination-prone trace element isotopes Cd, Fe, Pb, Zn, Cu, and Mo intercalibration. Limnology and Oceanography: Methods, 10: 653-665.

Conway, T.M., Sieber, M., Ellwood, M.J., Takano, S., Sohrin, Y. and Vance, D., 2018. CT31A-03: The competing influence of local cycling, regional sources and Southern Ocean processes in

influencing Fe isotope cycling at lower latitudes in the oceans, Ocean Sciences, Portland, Oregon.

Ellwood, M.J., Bowie, A.R., Baker, A., Gault-Ringold, M., Hassler, C., Law, C.S., Maher, W.A., Marriner, A., Nodder, S., Sander, S., Stevens, C., Townsend, A., van der Merwe, P., Woodward, E.M.S., Wuttig, K. and Boyd, P.W., 2018. Insights Into the Biogeochemical Cycling of Iron, Nitrate, and Phosphate Across a 5,300 km South Pacific Zonal Section (153°E–150°W). Global Biogeochemical Cycles: 2017GB005736.

Ellwood, M.J., Hassler, C., Moisset, S., Pascal, L., Danza, F., Peduzzi, S., Tonolla, M. and Vance, D., 2019. Iron isotope transformations in the meromictic Lake Cadagno. Geochimica et Cosmochimica Acta, 255: 205-221.

Reviewers' comments:

Reviewer #2 (Remarks to the Author):

For my last review, I was encouraged by the editor to present a complete and honest description of my concerns about the potential for inaccurate data when pushing Fe isotopes methods to report data on lower-Fe samples than previously reported.

I suggested that if the authors didn't want to perform more methods development, then they might:

"honestly discuss the incredible challenges of measuring Fe at such low concentrations, and acknowledge that they have not yet done the “gold standard” experiment of doping an isotope standard back into Fe-free seawater (as described above). They could also provide even more information about issues such as the variability in their blank. Then they could discuss the reasons why they still believe that their data are sound, and reasons why any expected small errors in their measurement should not undermine their basic conclusions."

In response they added two sentences stating that their methods perform well in intercalibration. In not adding more detail, I think the authors are missing an opportunity to move the field forwards in terms of understanding how far we can push Fe isotope measurements for low-Fe waters, which is extraordinarily important considering that such HNLC waters are exactly where Fe is a limiting nutrient. I don't mean to imply that the measurements presented here are somehow lagging behind the data quality of other labs (indeed their responses demonstrate that their data matches that of other top labs, see below), but just to point out that they are reporting d56Fe for waters with roughly an order of magnitude less Fe than anything else previously reported. In my opinion, discussing the challenges and successes of such measurements is an important and useful part of such a boundary-pushing effort.

While the authors have provided a great deal of additional information, which I appreciate, it doesn’t quite get to the heart of my concern. The authors have demonstrated that they can perform Fe isotope analyses with skill just as high as other international labs. And they have demonstrated through intercalibration that they are able to accurately measure d56Fe on relatively high-concentration (>~0.2 nM) samples. But I'm still not totally convinced that they (or anybody!) can accurately measure d56Fe on samples with tens-of-picomolar concentrations of Fe. All of the intercomparison exercises and plots they present deal with samples containing several hundred picomolar Fe. I would argue that the "platinum standard" intercomparison exercises are appropriate for showing that their methods are useful for hundreds-of-picomolar Fe, but that the "gold standard" re-doping experiments are really the only way to show whether or not it is possible to accurately measure d56Fe on tens-of-picomolar Fe. Of course, realizing the difficulty of such experiments, I also suggested that they might provide more detail in the manuscript about their method's possible strengths and weaknesses. With a little more detail, I think this manuscript will not only contribute the main points of their paper, but also contribute to an understanding of these meethods.

All that said, I do realize that we are now discussing tiny details about the discussion of methods in this manuscript. This is not central to the overall message and value of the work, which I appreciate, and I do honestly hope it can be published soon.

Seth

Dear Dr Frischkorn,
Below in blue text is our rejoinder to comments raised by reviewer 2.
Regards
Michael

Reviewer #2 (Remarks to the Author):

For my last review, I was encouraged by the editor to present a complete and honest description of my concerns about the potential for inaccurate data when pushing Fe isotopes methods to report data on lower-Fe samples than previously reported.

I suggested that if the authors didn't want to perform more methods development, then they might:

"honestly discuss the incredible challenges of measuring Fe at such low concentrations, and acknowledge that they have not yet done the “gold standard” experiment of doping an isotope standard back into Fe-free seawater (as described above). They could also provide even more information about issues such as the variability in their blank. Then they could discuss the reasons why they still believe that their data are sound, and reasons why any expected small errors in their measurement should not undermine their basic conclusions."

In response they added two sentences stating that their methods perform well in intercalibration. In not adding more detail, I think the authors are missing an opportunity to move the field forwards in terms of understanding how far we can push Fe isotope measurements for low-Fe waters, which is extraordinarily important considering that such HNLC waters are exactly where Fe is a limiting nutrient. I don't mean to imply that the measurements presented here are somehow lagging behind the data quality of other labs (indeed their responses demonstrate that their data matches that of other top labs, see below), but just to point out that they are reporting d56Fe for waters with roughly an order of magnitude less Fe than anything else previously reported. In my opinion, discussing the challenges and successes of such measurements is an important and useful part of such a boundary-pushing effort.

In response, we have added a paragraph to the manuscript highlighting the challenges to making iron isotope measurements on low concentration samples. see lines 335 to 365.

“As with all open ocean seawater work, during the collection and processing of samples contamination can hinder the production of accurate and meaningful data. The added challenge for Fe isotope studies, particularly for low concentration systems such as the Southern Ocean, is obtaining enough material for isotope analysis. For the result presented here, the dFe processing blank associated represents as much 20 ± 17% of the concentration and the isotope signal. While concentration uncertainties are highest for shallow samples collected in the CCE, the structure of the dFe concentration versus depth profile for this station, and indeed the other two stations, are oceanographically consistent, i.e. they have low surface water concentrations that increase with depth45. In a companion study, dissolved zinc concentration and zinc isotope results obtained from the same samples showed no indication of trace metal contamination associated with sample collection and processing46. For the dFe isotope results, there is also the added challenge of obtaining enough material for isotope analysis. Here we optimised the isotopic measurement of dFe by reducing the volume of each sample presented for analysis (0.3 to 0.35 mL) thereby upping its

concentration to reduce errors associated with instrument noise41,47. We also utilised a sample-spike ratio of ~1.6 (spike 57Fe-58Fe ratio = 1.05) such that counting errors are minimised for 56Fe, 57Fe, and 58Fe. Even with these steps, the influence of instrument noise increased for low concentration Fe samples (Figure S6). While the uncertainty window around these measurements is larger than that for samples with a higher dFe concentration, the upper water column variations for δ56Fediss between 15 and 150 m are statistically distinct and oceanographically consistent. The enrichment of δ56Fediss within the euphotic zone is consistent with measurements made at 32.5°S, 150°W (Figure S10) and other recent measurements for low dFe concentration waters of the Southern Ocean48. Likewise, the decline in δ56Fediss values below the euphotic zone is consistent with measurements made at 32.5°S, 150°W (Figure S10), although one should be mindful that this station is outside of the Southern Ocean such that the biological community leading to variation in δ56Fediss is likely to be different."

While the authors have provided a great deal of additional information, which I appreciate, it doesn't quite get to the heart of my concern. The authors have demonstrated that they can perform Fe isotope analyses with skill just as high as other international labs. And they have demonstrated through intercalibration that they are able to accurately measure d56Fe on relatively high-concentration (>~0.2 nM) samples. But I'm still not totally convinced that they (or anybody!) can accurately measure d56Fe on samples with tens-of-picomolar concentrations of Fe. All of the intercomparison exercises and plots they present deal with samples containing several hundred picomolar Fe. I would argue that the "platinum standard" intercomparison exercises are appropriate for showing that their methods are useful for hundreds-of-picomolar Fe, but that the "gold standard" re-doping experiments are really the only way to show whether or not it is possible to accurately measure d56Fe on
tens-of-picomolar Fe. Of course, realizing the difficulty of such experiments, I also suggested that they might provide more detail in the manuscript about their method's possible strengths and weaknesses. With a little more detail, I think this manuscript will not only contribute the main points of their paper, but also contribute to an understanding of these meethods.

We would like to point out that the intercalibration study actually covered the following concentration range 0.017 and 0.72 nmol $kg^{-1}$. Indeed for samples shallower than 150 m were below 100 pmol $kg^{-1}$ with the lowest being 17 pmol $kg^{-1}$. We have pointed this out too. That said, in the added paragraph (line 335 onwards), we highlight the challenges with the method in overcoming instrument noise which increases isotopic errors. We have also justified why we think our dissolve iron isotope dataset is reasonable.

All that said, I do realize that we are now discussing tiny details about the discussion of methods in this manuscript. This is not central to the overall message and value of the work, which I appreciate, and I do honestly hope it can be published soon.

Seth

# .Distinct iron cycling in a Southern Ocean eddy

Michael J. Ellwood*[1], Robert F. Strzepek[2], Peter G. Strutton[2,3], Thomas W. Trull[4], Marion Fourquez[2] and Philip W. Boyd[2].

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2. Institute for Marine and Antarctic Studies, University of Tasmania, Hobart, Australia

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**Abstract**

Mesoscale eddies are ubiquitous in the ocean, controlling ocean-atmosphere heat and gas exchange. However, their physical influence on phytoplankton production in the iron-limited Southern Ocean remains unknown. Here, we probed iron (Fe) cycling in a subantarctic cold-core eddy during the austral autumn. In-eddy, surface dissolved Fe concentrations and phytoplankton productivity and biomass were exceedingly low relative of external waters. In contrast, in-eddy phytoplankton Fe-to-carbon uptake ratios were elevated 2-6 fold, indicating upregulated intracellular Fe acquisition, thus reducing the residence time of Fe in the dissolved pool to ~1 day. Dissolved Fe isotope values were isotopically heavy in the euphotic zone, indicative of extensive trafficking of Fe by cells within the eddy. Below the euphotic zone, Fe isotope values were lighter and coincident with peaks in recycled nutrients and cell abundance, again indicative of enhanced microbially-mediated Fe recycling. Our measurements show that the isolated nature of eddies can produce distinctly different Fe biogeochemistry compared to surrounding waters with cells upregulating iron uptake and using recycling processes to sustain themselves. Recognising these eddy properties is essential to understanding how they contribute to the biophysicochemical structure of the Southern Ocean.

Words = 187

**Main text**

Mesoscale eddies are ubiquitous in the ocean[1,2] and play a crucial role in the transfer of heat, carbon and nutrients between the deeper ocean, surface waters and the atmosphere[3-6]. Cold-core eddies in the Southern Ocean are defined by strong clockwise rotation, cooler temperatures and negative sea-surface height anomalies[7,8]. These eddies can have closed circulation thus 'trapping' [ref 9] the biogeochemical properties of these features such that nutrient, chlorophyll and particle concentrations can be distinct relative to those in the surrounding waters[2,9-11]. They can transport these biogeochemical properties vast distances[12] thus they are important from an oceanographic point of view, especially if they cross water mass boundaries such as the Polar Front or the Subantarctic Front[7,11,13].

The concentration of dissolved Fe (dFe) in remote Southern Ocean surface waters, away from continental and island input sources, is typically sub-nanomolar (60-200 pmol $kg^{-1}$) [ref 14,15]. The lower limit for this dFe range is thought to be controlled by organic complexation and atmospheric supply. In the Southern Ocean, atmospheric inputs are very low and the supply of Fe usually is provided via

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upwelling and upward mixing of deeper iron-enriched waters[14,16]. However, eddies can become 'structurally closed' post-development, so their ability to entrain and detrain dFe, nutrients, phytoplankton and zooplankton can be restricted[1,17], thus making them a static 'mesocosm-like' environment. Structural breakdown of the eddy and the export of sinking organic matter are the principal biogeochemical loss terms. Our study focused on one such eddy to understand the biogeochemical cycling of Fe in this isolated structure. We contextualise our in-eddy Fe speciation (dissolved and particulate) and isotope measurements with primary productivity and biological observations and contrast them to external sites within the Subantarctic zone.

Between 30 March and 4 April 2016, we sampled a cyclonic cold core eddy that had spawned from the northern jet of the Subantarctic Front in mid-February 2016 [ref 3,4]. Satellite-derived sea surface temperature imagery, vertical profiles of temperature, salinity and nutrients, and undulating sensor measurements revealed that the eddy was biogeochemically distinct from the waters surrounding it at two reference stations (SAZ and SOTS; Figures 1, S1, S2 and Video S1). This eddy was typical with respect to its size (diameter) and life-span with that of other eddies in the region [3], however it was more intense in terms of rotational speed and amplitude. Sea surface temperatures in the eddy were about 2°C lower than surrounding waters, again more intense than basin-averaged anomalies of about 0.5°C [ref 1]. Chlorophyll concentrations were approximately 1.5 times lower within the eddy, consistent with the analysis of 'trapping' eddies[9,11]. Large differences in fluorescence and transmissivity with depth inside the eddy highlight low biological production at the time of sampling (Figure S1), which we confirmed with *in situ* biological measurements. In-eddy pico-plankton and nano-plankton cell numbers and primary productivity rates were reduced compared to external stations (Figures 2, S3 and S4). In-eddy primary productivity declined from 0.162 mmol C $m^{-3}$ $d^{-1}$ at 80% incident irradiance (15 m) to 0.011 mmol C $m^{-3}$ $d^{-1}$ at 1% incident irradiance (100 m). Outside of the eddy, primary productivity was higher, with values of 0.30 mmol C $m^{-3}$ $d^{-1}$ and 0.38 mmolC $m^{-3}$ $d^{-1}$ at 80% incident irradiance (15 m) at the SAZ and SOTS sites, respectively. This is consistent with productivity (~0.3 mmol C $m^{-3}$ $d^{-1}$) previously determined for the region[18], and about three times higher than in-eddy rates. These observations are all consistent with the eddy originating south of the northern extension of the Subantarctic Front where temperatures and chlorophyll concentrations were lower (Figure 1 and Video S1 )[4].

In the Southern Ocean, Fe limits phytoplankton production seasonally[16,18]. Our in-eddy dFe concentrations are amongst the lowest ever measured for the Southern Ocean, with values between 18 and 33 pmol $kg^{-1}$ for the upper 200 m (Figures 3 and S2). Because the eddy developed in the northern jet of the Subantarctic Front, the Fe status of the eddy likely reflects the initial

biogeochemistry of waters further south but modified on its northward transit. Indeed, nitrate and phosphate concentrations at 53°52'S; 147°16'E for waters near where the eddy formed were 2.3 μmol $L^{-1}$ and 0.1 μmol $L^{-1}$ higher, respectively than in-eddy levels suggesting biogeochemical modification of surface water properties as the eddy evolved. Interestingly, silicate levels were similar between the two sites suggesting that changes in nitrate and phosphate drawdown are likely facilitated by non-siliceous phytoplankton. Previous nearby summertime measurements for dFe from near where the eddy spawned, at and south of the Subantarctic Front, ranged between 70 and 210 pmol $kg^{-1}$, thus these low in-eddy dFe values appear to result from in-eddy processes (Table 1). Outside the eddy, dFe concentrations were 35 to 68 pmol $kg^{-1}$ and 57 to 210 pmol $kg^{-1}$ for the SAZ and SOTS stations, respectively (Figure 1), consistent with measurements for this region and the Southern Ocean generally (Table 1)[15]. Like dFe, in-eddy particulate Fe (pFe) concentrations were extremely low with values between 13 and 29 pmol $kg^{-1}$ for the upper 200 m (Figure 3). These low pFe concentrations reflect the low input of lithogenic material (dust) into the Southern Ocean (see supporting text)[19]. This observation is supported by the fact that between 51 and 69 % of the pFe pool in the upper 250 m of the eddy is considered biogenic (Figure S5).

Our results raise the question: How can dFe be consumed to such low concentrations by the in-eddy phytoplankton community, compared to external waters? We explored this question by measuring the rate of Fe uptake by cells inside and outside of the eddy. In-eddy Fe uptake rates were higher at 15 m (80% incident irradiance) compared to rates outside the eddy at the two reference sites (Figure 2 and S2). With depth, in-eddy Fe uptake rates decreased, but they were always higher than rates measured for the SAZ site (Figure 2). In-eddy carbon-normalised Fe uptake rates (expressed as a Fe:C ratio) for the resident phytoplankton community were approximately 4-fold higher compared to the two references sites and historical measurements for the region (Table 1). This is a surprising result considering the low in-eddy biomass at the time of occupation (Figures S1 and S3). A higher Fe:C uptake ratio for in-eddy phytoplankton is consistent with upregulation of cellular Fe acquisition machinery, to help acquire dFe. Indeed, Fe-limited phytoplankton have been shown to transport Fe into the cell at a faster rate than Fe-replete cells[20]. The structurally isolated nature of the eddy with respect to deep and surrounding waters (Figure S1) also reduced dFe replenishment from below and laterally. The diffusional supply of dFe into the euphotic zone (0-85 m) is estimated to be 0.25 nmol $m^{-2}$ $d^{-1}$ based on the dFe gradient between 200 and 70 m and an eddy diffusion coefficient of $K$ = 3 x$10^{-5}$ $m^2$ $s^{-1}$ (ref 16,21). This diffusional dFe supply rate is approximately 7 x$10^3$ times lower than the Fe utilisation rate of 1.88 μmol $m^{-2}$ $d^{-1}$ across the euphotic zone, thus highlighting the extreme nature of this cold core eddy with respect to Fe supply. It is possible that $K$ values in this region might be as high as $10^{-4}$ $m^2$ $s^{-1}$, but values higher than $10^{-3}$ $m^2$ $s^{-1}$ are never observed[22] and for the diffusive supply

and biological demand to match would require $K$ = 0.2 $m^2$ $s^{-1}$. Using the utilisation rate and a dFe inventory of 1.87 μmol Fe $m^{-2}$ for the euphotic zone, we estimate a residence time of 1 day for dFe. This short residence time indicates that Fe is being heavily trafficked within the euphotic zone between the dissolved pool and the microbial community. The increased importance of Fe recycling favours smaller phytoplankton cells, which is reflected in the cell abundances, the size-fractionated iron uptake and the Fe:C ratio datasets (Figures S3 and S4).

The elevated in-eddy Fe:C uptake ratios also raise the question as to how phytoplankton are enhancing dFe uptake. Enhanced dFe uptake can occur through a combination of processes[14], including increased production of Fe transporters on the surface of cells, a reduction in cell size, the production of Fe binding ligands, and the use of Fe(III) reductase proteins to enhance Fe(II) production and hence the acquisition of Fe from organic complexes. We used the isotopic composition of dFe and pFe to probe Fe uptake within the eddy.

The isotope composition of dFe ($\delta^{56}Fe_{diss}$) showed distinct variability with depth and between stations (Figure 3). In the euphotic zone (0-85m) of the cold core eddy, $\delta^{56}Fe_{diss}$ values are isotopically heavy at +1.19 ‰ and distinct ($p < 0.005$, T-test) to that of pFe ($\delta^{56}Fe_{part}$) and the $\delta^{56}Fe_{diss}$ composition for waters below the euphotic zone (0.00 ‰ at 150 m). The $\delta^{56}Fe_{diss}$ composition for surface waters at this site are also isotopically distinct ($p < 0.005$) to $\delta^{56}Fe_{diss}$ values measured at the SAZ and SOTS stations (Figure 3). At these stations, euphotic zone $\delta^{56}Fe_{diss}$ and $\delta^{56}Fe_{part}$ are isotopically similar to each other but significantly lower ($p < 0.005$) than in-eddy $\delta^{56}Fe_{diss}$ values (Figure 3).

The heavy in-eddy $\delta^{56}Fe_{diss}$ values for the euphotic zone are consistent with biological fractionation during dFe acquisition by phytoplankton. Modelling of the $\delta^{56}Fe_{diss}$ dataset using a closed system model produced isotope fractionation factors (ε) of -2.3 ‰ for samples collected in the euphotic zone (15 to 100 m; Figure 4). Interestingly, the in-eddy $\delta^{56}Fe_{part}$ values for the euphotic zone are not consistent with an instantaneous or an integrated isotope fractionation process associated with a closed system model. Generally, the expectation is that as dFe is consumed, the pFe pool should become isotopically heavier for both the instantaneous and the accumulated product. While $\delta^{56}Fe_{part}$ does appear to be subtly heavier with decreasing dFe concentration, it is not consistent with closed-system dependency for the biological reduction of Fe(III) to Fe(II) by cells (Figure 4). The physical cycling of $\delta^{56}Fe_{part}$ and $\delta^{56}Fe_{diss}$ offers a possible explanation for this discrepancy: $\delta^{56}Fe_{part}$ is distributed downward through the euphotic zone without general modification by sinking, whereas changes in the $\delta^{56}Fe_{diss}$ with depth (or dFe concentration) across the euphotic likely represent mixing across the euphotic zone.

Using a generalised 1-D model, we simulated these two different mechanisms over the seasonal cycle and found that the $\delta^{56}Fe_{diss}$ signal could be adequately modelled using isotope fractionation associated with just dFe uptake alone ($\varepsilon$ = -1 ‰) or a combination of isotope fractionation processes, namely dFe uptake ($\varepsilon$ = -0.6 ‰), pFe regeneration ($\varepsilon$ = +0.15 ‰), dFe scavenging from solution ($\varepsilon$ = -0.3 ‰) and dFe complexation to natural organic ligands ($\varepsilon$ = +0.6 ‰) (Figure 4 and S9, see supplementary material for extended discussion). The overall value for $\varepsilon$ is considerably smaller than the expected value (between -2 and -3 ‰ [ref 23]) for biological reduction of Fe(III) to Fe(II) by cells, suggesting that dFe isotope fractionation is likely associated with the rapid trafficking of Fe between the dissolved pool and cells (Figure 4). The recycling of Fe between pools may also amplify the $\delta^{56}Fe_{diss}$ signal. The model also produces $\delta^{56}Fe_{part}$ values within the range measured in the euphotic zone, supporting the idea that mixing and particle sinking are the primary mechanisms responsible for distributing the $\delta^{56}Fe_{diss}$ and $\delta^{56}Fe_{part}$ signals across the euphotic zone.

Iron bioavailability is known to influence the oxidation rates of ammonia and nitrite[24], such that both accumulate if Fe is limiting. An in-eddy minimum in $\delta^{56}Fe_{diss}$ (Figure 4) coincided with peaks in heterotrophic bacterial cell numbers (Figure S3) and nitrite concentration at 150 m, and just below the peak in ammonium concentration (Figure S5). This minimum in $\delta^{56}Fe_{diss}$ and the maxima in nitrite, ammonia and heterotrophic bacterial cell numbers provides evidence for Fe control of the microbial recycling of organic matter in the mesopelagic. Note that no localised minimum in $\delta^{56}Fe_{diss}$ or peaks in heterotrophic bacterial cell abundance or ammonium and nitrite occurred below the euphotic zone at the SAZ or SOTS stations (Figure S3 and S5) indicating that Fe limitation of the microbial component of the mesopelagic community is restricted to the eddy station (Figure S5). The model reveals seasonality in dFe and pFe concentrations suggesting that these signals of particulate Fe sinking and remineralization are initiated in spring and persist through to autumn (Figure S8), thus it is likely that their influence on Southern Ocean biogeochemistry may be even greater than suggested by the autumn observations presented here.

Satellite analysis of thousands of cyclonic eddies from the Southern Ocean reveals that they are generally cooler than surrounding waters but have higher chlorophyll concentrations in winter and spring and lower chlorophyll concentrations in summer and autumn[11,25]. These eddies have shallower mixed layers, particularly in winter and spring[25,26]. The increase in production during winter and spring likely results from an alleviation of light limitation[27], which is a key variable limiting plankton growth after winter mixing has reset the dFe concentration from levels that can limit phytoplankton production[16]. In contrast, shallow mixed layers during summer result in cyclonic eddies having a lower dFe inventory and hence low phytoplankton production compared to

surrounding waters[25]. Our study confirms that cyclonic eddies can have extremely low dFe inventories, thus requiring the resident plankton to upregulate iron uptake machinery and use recycling activities to sustain themselves. Such conditions favour small, non-siliceous cells, thus reducing export. At any time there are about 1200 eddies in the Southern Ocean, and approximately 50% of them are cyclonic[26], thus the extreme Fe limitation of Southern Ocean phytoplankton, as reported here, is likely to be the prevalent state during summer and autumn[25].

Main text 2239 words and 27 main references excluding Table 1.

## Methods

*Voyage*

The cold-core cyclonic eddy was studied between 28 March and 3 April 2016 and part of a GEOTRACES process study (Figure 1). The eddy was about 190 km in diameter and was a stable feature that had formed approximately one month prior to sampling (Figure 1, Video S1). It was formed by detaching from waters 2 degrees south near the Subantarctic Front[3,4]. Post formation, the eddy moved slowly northward across the northern extension of the subantarctic front (SAF-N; Video S1). As it moved, the eddy completed approximately 7 full rotations during is 109 day lifetime[3] The biogeochemical properties of the cold-core cyclonic eddy were contrasted with two other sites in the subantarctic zone. One was located at 51.90°S, 148.51°E, designated a Subantarctic zone site (SAZ) and the other at 46.77°S, 142.03°E, the Southern Ocean Time Series (SOTS) site (Figure 1).

*CTD, nutrient sampling*

Conductivity, temperature, and depth (CTD) profile data and water samples for nutrients and biological parameters were collected with a winch-lowered package consisting of an SBE 911plus CTD, a Turner Designs fluorometer, and a 24-bottle SBE 32 Carousel water sampler. Salinities were calibrated to standard seawater (International Association for the Physical Sciences of the Ocean). Samples for phosphate, nitrate, nitrite, ammonia and silicic acid were collected and analysed at sea on unfiltered samples using a Seal AA3 segmented flow system following the procedures outlined by Armstrong, et al. [28] and Wood, et al. [29].

*Trace metal Sampling*

Seawater samples for trace metal and isotope determination were collected using Teflon-coated, externally-sprung, 12-litre Niskin bottles attached to an autonomous rosette equipped with a Conductivity Temperature Depth (CTD) unit (SeaBird 911 plus, USA). Upon retrieval, the Niskin bottles were transferred into a clean container laboratory fitted with HEPA-filtered air workstations. Seawater samples for dissolved trace metal analysis were filtered through acid-cleaned 0.2-µm capsule filters (Supor AcroPak 200, Pall) and acidified with distilled nitric acid to a final pH ≤ 1.8. The sampling protocols followed recommendations in the 'GEOTRACES Cookbook' (http://www.geotraces.org/science/intercalibration/222-sampling-and-sample-handling-protocols-for-geotraces-cruises).

Particulate trace metal samples were collected *in situ* onto acid-leached 0.2-µm Supor (142 mm diameter) filters (Pall, Australia) using six large-volume dual-head pumps (McLane Research

Laboratories) deployed at various water depths. For most profiles, one pump depth was used as a blank check whereby only 4 L of water was pumped through the filter. This filter was then processed using the same procedure as the other samples.

*Primary Productivity and Iron uptake*

Net primary productivity and Fe uptake rates were determined for water column samples collected at six depths between 0 and 100 m [ref 30]. Water samples were collected pre-dawn from trace metal clean Niskin bottles deployed on a trace metal rosette. Sampling depths were determined from *in situ* irradiance depth profiles obtained during midday CTD casts the day prior to collection. Samples were dispensed into 300 mL acid-washed polycarbonate bottles and spiked with 16 µCi of Sodium $^{14}$C-bicarbonate ($NaH^{14}CO_3$; specific activity 1.85 GBq mmol$^{-1}$; PerkinElmer) and 0.2 nmol L$^{-1}$ of an acidified $^{55}$Fe solution ($^{55}FeCl_3$ in 0.1 M Ultrapure HCl; specific activity 30 MBq mmol$^{-1}$; PerkinElmer). Six samples (5 light and one dark bottle per irradiance) were incubated for 24 hours in a deck-board incubator under natural sunlight at 6 light intensities (from 80 to 1.0 % of incident irradiance). Light attenuation was adjusted by varying the layers of neutral density mesh and measured with a Biospherical Instruments QSL2101 Quantum Scalar PAR Sensor. The temperature of the incubator was controlled by a continuous supply of surface seawater.

Upon completion of the 24-hour incubation, 4 replicate samples were serially vacuum-filtered (<10 mm Hg) through 20 µm, 2.0 µm, and 0.2 µm porosity polycarbonate filters (47 mm diameter; Poretics) separated by 200 µm nylon mesh. Two size-fractionated samples were washed with Titanium(III) EDTA – citrate reagent for 5 min to dissolve Fe (oxy)hydroxides and remove ferric ions bound to the outer membrane surface[31], and rinsed three times with 15 mL of 0.2 µm-filtered seawater [32,33] and the other two size-fractionated samples were rinsed only with 0.2 µm-filtered seawater. In addition, two samples were filtered through 0.2 µm filters (a total community 'light' control, and a total community 'dark' control). Data for the dark-corrected, size-fractionated samples rinsed with the Ti(III) EDTA – citrate reagent are reported here (i.e., intracellular Fe:C uptake ratios).

The filters were transferred to 20 mL scintillation vials (Wheaton) and acidified with 100 µL of 1.0 M HCl to volatilize any remaining inorganic carbon[30]. Samples were counted on a liquid scintillation counter (PerkinElmer Tri-Carb 2910 TR) with a dual-label counting protocol after the addition of 10 mL of liquid scintillation cocktail (UltimaGold, PerkinElmer). Unfiltered water samples (1 mL) were used to quantify the concentrations of added $^{14}$C and $^{55}$Fe, and Fe:C uptake ratios for the size

fractions were calculated from specific activities after accounting for both added and ambient dissolved Fe concentrations.

*Cell counts*

Flow cytometric analyses were performed following protocols outlined by Marie, et al. [34]. Samples for cell counts were preserved with glutaraldehyde and stored frozen at -80 °C to protect against cell lysis and the loss of autofluorescence. Prior to analysis, frozen samples were rapidly thawed in a water bath at 70 °C for 3 min and aliquots were taken for autotrophic and prokaryote cell counts. Sample aliquots were kept on ice in the dark and promptly analysed on a Becton Dickinson FACScan flow cytometer fitted with a 488 nm laser. Milli-Q water was used as sheath fluid for all analyses. Before and after each run, samples were weighed to determine the amount of sample analysed.

Autotrophic cell abundance samples were prepared by pipetting 1 mL of sample to a clean 5 mL polycarbonate tube, with 2 µL of PeakFlow Green 2.5 µL beads (Invitrogen) added as an internal fluorescence and size standard. Each sample was run for 5 min at a high flow rate of 40 µL $min^{-1}$. Autotrophic cell populations were separated into regions based on their chlorophyll autofluorescence in red (FL3) versus orange (FL2) bivariate scatter plots. *Synechococcus* sp cells were determined from their high FL2 and low FL3 fluorescence. Pico- (<2 µm) and nanophytoplankton (2-20 µm) communities were determined from their relative cell size in side scatter (SSC) versus FL3 fluorescence bivariate scatter plots.

Samples for prokaryote cell abundance were prepared by pipetting 1 mL of sample to a clean 5 mL polycarbonate tube. Samples with high prokaryote cell counts were diluted to 1:10 with 0.2 µm filtered seawater (FSW) to remove underestimation of cell concentration from coincidence (100 µL sample in 900 µL FSW). Cells were stained for 20 min with 5 µL of SYBR Green I (Invitrogen) at a final dilution of 1:10,000. An additional 2 µL of PeakFlow Green 2.5 µL beads (Invitrogen) were added to the sample as an internal fluorescence and size standard. Each sample was run at a low flow rate of ~12 µL $min^{-1}$ for 3 min and prokaryote cell abundance was determined from bivariate scatter plots of SSC versus green (FL1) fluorescence.

*Iron isotope analysis*

Particulate samples for trace element and $\delta^{56}$Fe determination were thawed and processed using previous acid digestion protocols[35-37]. For dFe isotope determination, seawater samples (2L) were spiked with a $^{57}$Fe-$^{58}$Fe double spike (1-3:1 spike: sample ratio)[38,39]. Samples were left overnight to equilibrate, after which they were buffered to a pH of 4.5 with a trace-metal clean ammonium

283 acetate buffer and then passed over 0.5 mL columns packed with Nobias PA Chelate PA1L resin
284 (Hitachi-Hitec, Japan) at a flow rate of 2 mL $min^{-1}$. Samples were rinsed with 4 mL of ammonium
285 acetate buffer solution (1% w $w^{-1}$) followed by elution with 4 mL of 1 mol $L^{-1}$ nitric acid. Samples
286 were evaporated to dryness and redissolved with 0.5 mL of 6 mol $L^{-1}$ hydrochloric acid containing
287 $H_2O_2$. Samples were further purified using an anion exchange procedure similar to that described by
288 Poitrasson and Freydier [40]. Precleaning of the AG-MP1 resin involved rinsing with methanol, multiple
289 washes with 6 mol $L^{-1}$ HCl and 0.5 mol $L^{-1}$ $HNO_3$ before storage in dilute $HNO_3$. When required ~200
290 µL columns filled with the precleaned anion exchange resin AG-MP1 (Bio-Rad), conditioned by
291 washing with 0.5 mol $L^{-1}$ HCl, 0.5 mol $L^{-1}$ HNO3, Milli-Q water and finally 6 mol $L^{-1}$HCl before use.
292 Between use, columns were stored filled in 2% w $w^{-1}$ $HNO_3$. Columns were typically used 5-8 times
293 before being refilled with new precleaned resin. After sampling loading, salts and other elements not
294 of interest were eluted from the column by passing 3x 1 mL of 6 mol $L^{-1}$ hydrochloric acid. Iron was
295 eluted with 3x 1 mL of 0.5 mol $L^{-1}$ hydrochloric acid and evaporated to dryness. Samples were
296 redissolved in either 0.30 or 0.35 mL of 2% (w $w^{-1}$) nitric acid. The blank associated with the anion
297 exchange separation was 0.39 ± 0.34 ng (n = 4). Procedural concentration blanks for the whole
298 process were determined by passing small volumes (~50 mL) of an in-house seawater standard with
299 a concentration 0.78 ± 0.08 nmol $kg^{-1}$ over the Nobias PA Chelate PA1L resin and then through the
300 whole elemental and Fe isotope separation procedure. The dissolved iron concentration for this
301 smaller seawater was then scaled to 2L thus allowing us to estimate the blank associated with the
302 buffering of the sample, passing it over the Nobias PA Chelate PA1L resin and then over the anion
303 exchange columns. The blank associated with this test was determined to be 0.40 ± 0.32 ng (n = 5).
304 Note that we were not able to determine the isotope composition of the blank associated with the
305 extraction and processing procedure, so the isotope values presented in have not been blank
306 corrected. For dissolved samples, the total amount of Fe analysed ranged between 2 and 63 ng, thus
307 the contribution of the blank to the lowest concentration samples could have been between as
308 much 20 ± 17% of the lowest $\delta^{56}Fe_{diss}$ signal, i.e. for samples collected from the upper water column
309 within the CCE.

310 Iron isotopes were determined using a Neptune Plus multi-collector ICPMS (ThermoScientific) with
311 an APEX-IR introduction system (ESI, USA) and with X-type skimmer cones. Samples were measured
312 in high-resolution mode with $^{54}Cr$ interference correction on $^{54}Fe$ and $^{58}Ni$ interference correction on
313 $^{58}Fe$. Fe isotope ratios ($^{56}Fe/^{54}Fe$) ratios are reported in delta notation (‰) relative to the IRMM-014
314 Fe isotope reference material (IRMM, Brussels) using the double spike ($^{57}Fe$-$^{58}Fe$) correction
315 technique[38,39] where:

$$\delta^{56}Fe = \left( \frac{^{56}Fe/^{54}Fe_{sample}}{^{56}Fe/^{54}Fe_{IRMM-014}} - 1 \right) \text{x } 1000 \quad (1)$$

The overall instrumental error for dFe and pFe samples ranged between ± 0.04 ‰ and ± 0.64 ‰ (2σ). For low concentration samples, the instrumental error increased with decreasing Fe concentration and was associated with instrumental noise (Figure S6)[41]. Multiple large volume (3x 2L) extractions and analysis of an in-house seawater standard had a reproducibility of 0.76 ± 0.07 ‰ (mean ± 2x standard deviation). Multiple analysis of a particulate sample had a reproducibility of 0.15 ± 0.06 ‰ (mean ± 2x standard deviation). Analysis of geological samples NOD-A-1 and BCR-2 produced values of -0.43 ± 0.03 ‰ and 0.04 ± 0.07 ‰ (mean ± 2x standard deviation), respectively, which were consistent with literature values of -0.42 ± 0.07 [ref 42] for NOD-A-1 and 0.03 ± 0.06 [ref 42] for BCR-2. The performance of the Fe isotope method was also assessed through an intercalibration exercise for samples from the GP13 and GP19 GEOTRACES campaigns at a crossover station located at 30°S; 170°W. The iron isotope results from the exercise were comparable – i.e., the trends seen in the GP13 and GP19 profiles are consistent with each other covering a dFe range between 0.017 and 0.72 nmol $kg^{-1}$ (Figure S10) [43]

Dissolved Fe concentration for each sample was calculated using sample weight and the amount double spike added to the sample. This calculation is based on isotope dilution using the known proportion of $^{58}Fe$ in the $^{57}Fe$–$^{58}Fe$ double spike[38,44]. Note that the dFe concentrations presented here were not blank corrected, thus, they represent an upper concentration bound.

As with all open ocean seawater work, during the collection and processing of samples contamination can hinder the production of accurate and meaningful data. The added challenge for Fe isotope studies, particularly for low concentration systems such as the Southern Ocean, is obtaining enough material for isotope analysis. For the result presented here, the dFe processing blank associated represents as much 20 ± 17% of the concentration and the isotope signal. While concentration uncertainties are highest for shallow samples collected in the CCE, the structure of the dFe concentration versus depth profile for this station, and indeed the other two stations, are oceanographically consistent, i.e. they have low surface water concentrations that increase with depth[45]. In a companion study, dissolved zinc concentration and zinc isotope results obtained from the same samples showed no indication of trace metal contamination associated with sample collection and processing[46]. For the dFe isotope results, there is also the added challenge of obtaining enough material for isotope analysis. Here we optimised the isotopic measurement of dFe by reducing the volume of each sample presented for analysis (0.3 to 0.35 mL) thereby upping its

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concentration to reduce errors associated with instrument noise[41,47]. We also utilised a sample-spike ratio of ~1.6 (spike $^{57}Fe$-$^{58}Fe$ ratio = 1.05) such that counting errors are minimised for $^{56}Fe$, $^{57}Fe$, and $^{58}Fe$. Even with these steps, the influence of instrument noise increased for low concentration Fe samples (Figure S6). While the uncertainty window around these measurements is larger than that for samples with a higher dFe concentration, the upper water column variations for $\delta^{56}Fe_{diss}$ between 15 and 150 m are statistically distinct and oceanographically consistent. The enrichment of $\delta^{56}Fe_{diss}$ within the euphotic zone is consistent with measurements made at 32.5°S, 150°W (Figure S10) and other recent measurements for low dFe concentration waters of the Southern Ocean[48]. Likewise, the decline in $\delta^{56}Fe_{diss}$ values below the euphotic zone is consistent with measurements made at 32.5°S, 150°W (Figure S10), although one should be mindful that this station is outside of the Southern Ocean such that the biological community leading to variation in $\delta^{56}Fe_{diss}$ is likely to be different.

*Iron isotope modelling*

The closed system equation for the isotopic evolution of dFe as it is consumed can be described as follows:

$$\delta^{56}Fe_{dissolved} = \delta^{56}Fe_{dissolved.100m} + \varepsilon \ln(f) \quad (2)$$

Where ε represents the isotope enrichment factor between the product (biologically utilised Fe) and the substrate (dFe) and *f* presents the fraction of dFe relative to the concentration of dFe at 100 m. The evolution of the instantaneous or integrated isotope fractionation processes can be modelled using the following expressions:

$$\delta^{56}Fe_{particulate} = \delta^{56}Fe_{dissolved} - \varepsilon \quad (3)$$

$$\delta^{56}Fe_{particulate} = \delta^{56}Fe_{dissolved} + \frac{\varepsilon \ln(f)}{1-f} \quad (4)$$

*1D biogeochemical modelling*

The potential processes that influence the distribution and isotope fractionation of dFe and pFe were explored using a 1D model (Figure S7). The rationale for using this 1D model is to explore the relative influence (and interplay) of processes such as phytoplankton utilisation of Fe, its

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complexation to natural organic ligands, its regeneration from sinking organic matter and the role of scavenging on distribution and expression of isotope profiles. The model is based on Schlosser, et al. [49] and includes one phytoplankton group and references key nutrients including nitrate, phosphate and Fe (Figures S7, S8 and S9). The model includes mixing, which supplies nutrients into the euphotic zone and the main loss process for nutrients and Fe from the euphotic zone (organic matter export). The Fe component in the model also includes complexation to natural organic ligands, scavenging and the atmospheric supply of Fe through the deposition and dissolution of dust (Support Information, Figure S7)). The model also does not include advection, which we justify for several reasons: i) vertical advection, i.e. upwelling, occurs in the Southern Ocean primarily south of the Polar Front and not in the SAZ and SAF regions examined here for the cold core eddy[50], ii) latitudinal advection supplies waters with similar properties from upstream in the Antarctic Circumpolar Current [51], and can thus be ignored; and iii) transport is dominated by northward Ekman transport, and while this does supply nutrients over the annual mean, in late summer surface concentrations between the SAF and PFZ are very uniform [52], so this term can also be neglected. The equations and values associated with each biogeochemical process are presented in the Supporting materials and in Tables S1 and S2.

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**Author contributions**

M.J.E. and P.W.B. conceived the study with input from P.G.S. and T.W.T. on appropriate oceanographic sampling sites. M.J.E., R.F.S. and M.F. collected and analysed samples. M.J.E. wrote the paper with assistance from all co-authors.

## Data availability

The main datasets supporting the findings of this study are available within this article and its Supplementary Information, and additional data are available from the corresponding author on request.

**Table**

**Table 1.** Intracellular iron:carbon (Fe:C) ratios, Fe uptake rates and dissolved Fe concentrations for upper Southern Ocean waters between longitudes 142°E and 172°E.

| Station/Study Latitude | Fe:C ($\mu$mol mol$^{-1}$) | Fe uptake rate (pmol L$^{-1}$ d$^{-1}$) | Dissolved Fe (pmol kg$^{-1}$) | Notes | Reference |
|---|---|---|---|---|---|
| Cold core eddy - 49.7°S | 224 ± 38 | 25 ± 5 | 23 ± 1 | 15-40 m - 80-20% irradiance | this study |
| SAZ - 51.4°S | 40 ± 8 | 12 ± 2 | 48 ± 17 | 15-40 m - 80-20% irradiance | this study |
| SOTS - 46.7°S | 61 ± 7 | 28 ± 6 | 60 ± 4 | 15-40 m - 80-20% irradiance | this study |
| SOIREE - 61°S | 3-7.5[#] | 3.07[#] | 80 ± 30[$] | Fe enrichment experiment | Bowie et al. [53] |
| FeCycle - 46°S | 5.5 - 19 | 290-360 | 51 ± 11 | Subantarctic experiment | McKay et al. [54] |
| SOFex - 56°S | 9-11 | not reported | 140 | Northern Patch | Twining et al. [55] |
| SAZ Project - 47°S | 52 | 60 | 70 | SOTS station | Boyd et al. [18] |
| SAZ Project - 54°S | 78 | 55 | 70 | Polar water station - equivalent to where the cyclonic eddy originated | Boyd et al. [18] |
| SAZ-Sense P1 - 46.3°S | 70 ± 44 | 110 ± 10 | 260 ± 40 | Equivalent to SOTS station | Bowie et al. [56] |
| SAZ-Sense P2 - 54°S | 60 ± 9 | 34 ± 5 | 210 ± 20 | Polar water station - equivalent to where the cyclonic eddy originated | Bowie et al. [56] |
| SAZ-Sense P3 - 45.5°S | 74 ± 47 | 77 ± 10 | 440 ± 70 | Site close to Subtropical convergence | Bowie et al. [56] |

[$] Background dissolved Fe concentration before Fe infusion. [#] Fe:C and Fe uptake rates are for days 1-3 after Fe infusion.

**Figure captions**

**Figure 1.** Chlorophyll and sea surface temperature in the study area. **a** and **b**, Maps of chlorophyll *a* concentration **c**. Sea Surface Temperature (SST) and **d.** Sea Level Anomaly (SLA) for Southern Ocean waters south of Australia. The diamonds represent the Cold Core eddy station (CCE), the Subantarctic zone station (SAZ) and the Southern Ocean Time Series station (SOTS) and the solid black line represents the Triaxus tow (Figure S1). The chlorophyll *a* satellite data represents a monthly average for March 2016. The SST data are for 3 April 2016 and SLA data are for 25 March 2016. In **b** and **c** the contours lines are SST. Data extracted from https://coastwatch.pfeg.noaa.gov/erddap/griddap/.

**Figure 2.** Depth profiles of Fe and carbon uptake. Profiles of **a**, Fe and **b**, carbon uptake versus depth. **c**, Profiles of the intracellular Fe:C uptake ratio for the CCE, SAZ and SOTS stations.

**Figure 3.** Depth profiles of Fe concentration and isotope composition. Upper ocean (0 to 600 m) depth profiles of dFe and pFe concentration (**a, b, c**), and the isotope composition (**d, e, f**) for samples collected at the **a.**, **d.,** CCE, **b.**, **e.,** SAZ and **c.**, **f.,** SOTS stations. See the Methods section for details on the issues and challenges involved with measuring $\delta^{56}$Fe on samples with very low dFe concentrations.

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**Figure 4.** Iron isotope fractionation model calculations. **a.** Dissolved $\delta^{56}$Fe values for samples collected at the CCE, SAZ and SOTS stations versus Fe concentration. **b**. Model curves for closed steady-state isotope fractionation (equations 2 to 4) of dFe for the CCE. The best fit $\varepsilon$ value for the cold core eddy dFe data is -2.3 ‰. One-D model profiles (blue lines) for dFe and pFe versus depth for **c.** $\varepsilon$ equal to -1.0 ‰ ($\alpha_{uptake}$ = 0.999) and **d.** $\varepsilon$ equal to -2.3 ‰ ($\alpha_{uptake}$ = 0.9977) along with profiles of dFe and pFe isotope composition versus depth for the CCE.

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REVIEWERS' COMMENTS:

Reviewer #2 (Remarks to the Author):

I thank the authors for their diligence in addressing my concerns, and I think this manuscript is suitable for publication.

Dear Dr Frischkorn,
Below in blue text is our rejoinder to comments raised by reviewer 2.
Regards
Michael

Reviewer #2 (Remarks to the Author):

I thank the authors for their diligence in addressing my concerns, and I think this manuscript is suitable for publication.

Thank you, your time and efforts in reviewing our manuscript is appreciated!