Prof. Gerhard Herndl,

Associate Editor,
Biogeosciences

Monday, June 3, 2018

Dear Prof. Herndl,

Response to reviewers for manuscript bg-2017-551

On behalf of my co-authors and myself, we would like to thank both you and the reviewers for the responses in commenting upon our manuscript entitled “Seasonal development of iron limitation in the sub-Antarctic zone”. All comments were appreciated and have been taken into full consideration when making amendments to the revised manuscript submitted here.

We feel we have adequately addressed the reviewer’s comments and hope this manuscript is now acceptable for publication in Biogeosciences. We outline our response to each of the reviewer’s comments below.

Anonymous Referee #1

This manuscript presents an attempt to evaluate the seasonal cycle of iron stress in the sub-Antarctic zone using three bottle-scale iron enrichment experiments conducted in December–February. The novel aspect of the study is the reoccupation of the experimental site over 2 months. Whilst these observations cannot, by some margin, be used to confidently state overarching changes in SAZ iron stress in these months, they are still valuable to the scientific community and worthy of publication in Biogeosciences. However, I have a number of comments that should be addressed prior to publication. In particular, I think the authors should more carefully/critically evaluate how far their experiments can actually be used to evaluate the seasonal development of Fe limitation in the sub-Antarctic zone without an analysis of supporting depth-resolved Fe, mixed layer depths, and PAR data. Upon reflection of
the former, some rephrasing of the manuscript is required. Some additional
important method details are also lacking.

We agree with the reviewer that some of the conclusions were too strong for
what the data presented could tell us in regards to iron supply mechanisms
across the seasonal cycle. Specific sections in the text have been amended to
now refer to potential phytoplankton growth and productivity.

Some examples of how the conclusions have been adjusted are provided
below.

Line 25: “Here we demonstrate that at the beginning of the growing season,
there is sufficient iron to meet the demands of the phytoplankton community,
but that as the growing season develops the mean iron concentrations in the
mixed layer decrease and are insufficient to meet biological demand.”

Line 434: “Irrespective of the different supply mechanisms; winter-
entrainment, storm driven entrainment, diapycnal diffusion, photochemical
reduction or microbial regeneration, the iron supply to the mixed layer is not
sufficient for phytoplankton to reach to reach maximum growth potential and
completely drawdown all available macronutrients.”

Additionally, in table 1 depth-resolved mean concentrations of all nutrients
(including DFe) have been added which show seasonal depletions across all,
except for nitrate. The reasons for this are explicitly discussed below in
response to specific comments.

The paper is in general well written and referenced and the figures and tables are
clear and complete. My comments below are listed in order through the manuscript,
not by importance.

Specific comments:

Line 18: Variability in iron supply also includes dust (not mentioned)

Atmospheric deposition has been added as a potential source of iron supply
in the abstract now.

Line 21: “The variability in iron availability is due to an interplay between
winter entrainment, diapycnal diffusion, storm-driven entrainment,
atmospheric deposition, iron scavenging and iron recycling processes.”

Line 21–22: ‘incubation experiments were used to determine the importance of (iron)
supply mechanisms’. Can they really be used for this? All they actually indicate is the
biological response to incubation, not sources? A thorough analysis of Fe supply and
demand, in conjunction with the bioassays, would be needed to do this (only 3 Fe
values are reported).
I agree that this statement is misleading, and the incubation experiments cannot determine the importance of the supply mechanisms. As such this sentence has been modified.

Line 22: “Biological observations utilising grow-out iron addition incubation experiments were performed at different stages of the seasonal cycle within the SAZ to determine whether iron availability at the time of sampling was sufficient to meet biological demands at different times of the growing season.”

Line 26–27: The results presented do not support the claim of progressive Fe depletion - phytoplankton appear to respond more to the Fe later in summer, but the in-situ Fe concentrations stay the same.

Whilst the in situ concentrations at the specific experimental sampling depth do not change throughout the season, the mean values across the mixed layer and euphotic zone do change. Sections describing these changes have been added to the result and discussion where necessary, with the mean DFe concentrations in the mixed layer added to table 1. The specifics of the seasonal changes in the Fe inventory will be discussed in a companion paper (Mtshali et al., In Prep).

Line 239: “Mean silicate concentrations in the mixed layer were considered limiting and decreased between experiments (1.49 - 0.84 μM), while phosphate and dFe also displayed a gradual seasonal depletion (0.77 - 0.65 μM and 0.22 - 0.09 nM respectively); whereas nitrate concentrations increased throughout the growing season (10.41 - 12.92 μM) (Table 1).”

Line 72–73: Whilst excess nitrate+nitrite excretion under Fe stress may play a role, I think most would argue that high rates of resupply (relative to inefficient biological removal) overwhelmingly control the elevated residual nitrate in the Southern Ocean.

I agree with the comment here, that the excess nitrate is controlled by high rates of resupply. As such, the sentence has been modified to include both statements as below.

Line 76: “The result of this is excretion of excess nitrate and nitrite back into the water column, which combined with the high rates of resupply relative to biological uptake, can culminate in HNLC conditions typical of the Southern Ocean.”

Line 97: ‘were’ change to ‘that were’?

Were has been changed to that were - see line 102.

Line 99: Do the results actually indicate a change the photosynthetic efficiency? As the authors say themselves, most of the Fv/Fm change could well be due to pigment changes that have little to do with PSII efficiency.
This sentence has now been changed to reflect more broad changes in photophysiology, with what the specific changes mean to be left until the discussion.

Line 101: “This was done through a series of ship-board grow-out nutrient addition incubation experiments that were performed to determine the extent to which the addition of iron at different times of the growing season would relieve the phytoplankton from iron limitation driving changes in photophysiology, chlorophyll-a biomass and growth potential.”

Line 99: Change ‘biomass’ to ‘chlorophyll-a biomass’?

Biomass has been changed to chlorophyll-a biomass - see line 104.

Line 113: Table ‘X’?

I have moved the reference to table 1 as this could lead to confusion from the reader where table 2 has the results of the changes in biomass. Table 1 is now referred to on line 117 and its reference deleted from line 107.

Line 115: CTD abbreviation defined?

Line 120: “trace metal clean CTD (Conductivity Temperature Depth) rosette system”

Line 122: Why was water ‘allowed to settle’ in Go-Flo samplers? To increase the overall phytoplankton concentrations in the incubation bottles?

Apologies this section was misleading, the reason for not sampling the GoFlo bottles immediately is to allow the air circulation system of the container to filter out particles that may have entered the container when transferring the GoFlo bottles from the rosette, thereby reducing any potential contamination risks. As such I have removed this phrase from the sentence so that it now reads:

Line 127: “Water for experiments were transferred unscreened into acid-washed 50 L LDPE carboy (Thermo scientific) to ensure homogenization”

Section 2.2: - More details on the incubation experiment setup needed: What was the actual incubator? A culture cabinet? Something custom built? Please give details. - How does the PAR values supplied in the incubator differ from in-situ values? For this the authors will need to calculate an average ML PAR using their observations of ML, CTD PAR and ship-instrument PAR. Although this might be a pain, it might really help to pick apart the difference in growth environments experienced by the community just prior to incubation, and thereby help to interpret their response to the altered conditions.

The incubator was a modified fridge that was fitted with adjustable LED light strips with time control along with a cooling fan for temperature control, the brand of the incubator is Minus40 Specialised Refrigeration.
Line 141: “All incubations were performed within customised Minus40 Specialised Refrigeration™ units, which were fitted with adjustable (intensity and timing) LED strips as well as a thermostat and cooling fan for temperature control.”

We thank the reviewer for their suggestions to include PAR. A new row has been added to table 1 that includes the mean ± standard deviation mixed layer PAR calculated for the day of initiation from the co-located glider deployment. The light environment in the incubator was more closely matched to the average PAR in experiments 2 and 3 (~6.5 mol photons m\(^{-2}\) d\(^{-1}\)), as opposed to experiment 1 where it was on ~12 mol photons m\(^{-2}\) d\(^{-1}\) lower than the average mixed layer PAR.

The following text has been added to the discussion section to highlight in situ versus incubator PAR in the experiments:

Line 330: “The total daily PAR in the incubators ranged from 6.52 - 6.99 mol photons m\(^{-2}\) d\(^{-1}\), which is in good agreement for the in situ light environments of experiments 2 and 3. However, this was a ~62% decrease in the daily PAR that the phytoplankton community in experiment 1 were previously subjected to. Such a decrease in PAR would be expected to lead to a decrease in the downregulation of PSII by photodamage, coincident with an anticipated response in community structure. This could explain the observed increase in \(F_v/F_m\) and decrease in \(\sigma_{PSII}\), as larger cells tend to have a higher \(F_v/F_m\) and small \(\sigma_{PSII}\) in comparison to smaller cells (Suggett et al., 2009). Indeed we did observe a change in the community structure for experiment 1 (Fig. S2), suggestive that a decrease in light pressure resulted community response in the control treatment. However, the lack of taxonomic data at 72 h makes it difficult to distinguish whether the primary driver of this response is physiological, taxonomic or a combination of both.”

Lines 130-131: Which experiments were in duplicates and which in triplicates? Figure 2 states n=3 or n=5, so I do not understand this. Please clearly indicate number of biological replicates (number of bottles with the same treatment) and technical replicates (i.e. FRR/chl measurements made from the same bottle).

During each experiment both treatments had 16 bottles, whilst some bottles were sampled at time points for key parameters (i.e. nutrients, chlorophyll-a, FRRf), some bottles were terminated at specific timepoints to collect large volume samples for HPLC. No technical replicates were performed on the same bottle. This has been clarified with the following additional text:

Line 135: “Experiment incubations were conducted as biological replicates with 16 bottles per treatment for each experiment, these were sub-sampled at set time points for key variables as outlined in the Supplementary Information Table S1.”

A sub-sampling table has been added to supplementary information to explain in greater detail the specific sampling strategy.
<table>
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<th>Experiment</th>
<th>Variable</th>
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<th>48</th>
<th>72</th>
<th>120</th>
<th>144</th>
<th>168</th>
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</thead>
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<td>+Fe = 16 bottles</td>
<td>FRRf</td>
<td>3</td>
<td>3</td>
<td>n/a</td>
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<td>n/a</td>
<td>6</td>
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<td></td>
<td>Chl-a</td>
<td>3</td>
<td>3</td>
<td>n/a</td>
<td>3</td>
<td>3</td>
<td>n/a</td>
<td>6</td>
</tr>
<tr>
<td>Control = 16 bottles</td>
<td>Nutrients</td>
<td>3</td>
<td>3</td>
<td>n/a</td>
<td>3</td>
<td>3</td>
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<td></td>
<td>HPLC</td>
<td>3</td>
<td>n/a</td>
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<tr>
<td>+Fe = 16 Bottles</td>
<td>FRRf</td>
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<tr>
<td></td>
<td>Chl-a</td>
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<td>3</td>
<td>n/a</td>
<td>3</td>
<td>5</td>
<td>n/a</td>
<td>10</td>
</tr>
<tr>
<td>Control = 16 Bottles</td>
<td>Nutrients</td>
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<td>n/a</td>
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</table>
**Table S1**: Sub-sampling strategy for biological replicates of variables measured within each experiment. The number of samples collected for each variable at each timepoint is listed, where samples that were not collected is denoted by ‘n/a’.

Line 133-134: Can this approximate time of sub-sampling be included in Table 1? This might help to interpret the results (for instance, if daytime, the Fv/Fm increase in Exp 1 due to differences in PSII damage/down regulation between days).

A new row has been added to table 1 to include the initiation times, which were 07:00 for experiment 1, 20:00 for experiment 2 and 02:00 for experiment 3. In addition, text interpreting the results with respect to PAR has been address with specific reviewer comments below.

Also see line 330 in the manuscript for the specific text that addresses the effect of PAR in interpreting the results.

Line 144: Exactly how were the dFe samples filtered (method, on-ship/land)?

The DFe samples were filtered and analysed on land. This has been clarified with the following text:

Line 154: “Dissolved iron samples (DFe) were filtered through 0.2 µm cartridge filters (Acropack) equipped with a 0.45 µm pre-filter, drawn into acid washed 125 mL LDPE bottles (Nalgene, Thermoscientific), acidified with 30% HCl suprapur to pH ~1.7 (using 2 mL L⁻¹ criteria), double bagged and stored at room temperature until analysis on land at the Université de Bretagne Occidentale (UBO), France using the Chemiluminescence – Flow Injection Analyser (CL-FIA) method (Obata et al., 1993; Sarthou et al., 2003).”

Line 154: Was the instrument a FastTrackAll?

The instrument was a FastOcean integrated with a FastAct laboratory system attached as indicated in text – line 166.

Line 168: Was the FastPro software used or was custom code used? If the latter please give details.

  The FastPro software (v1.0.55) was used and clarified in the text.

Line 180: “Data from the FRRF were analysed to derive the fluorescence parameters as defined in Roháček (2002), by fitting transients to the model of Kolber et al. (1998) using the FastPro8 software (v1.0.55).”

Section 2.8: What was the test for means comparison between treatments? T-test?
The test for comparison between treatments and time was ANOVA - analysis of variance. This has been clarified in the text - Line 229.

“Sample means and standard deviations were calculated using Python, followed by tests for normality and equal variance prior to analysis of variance (ANOVA) to determine treatment effects (SciPy v0.17.1, Python v3.6). Significant results are reported at the 95% confidence level ($p < 0.05$).”

Lines 303-304: ‘The rapid increase in Fv/Fm in both treatments at 24 h is likely due to bottle effects i.e. a change in light environment.’ - This experiment needs discussing in more detail as the community response is clearly compatible with relief of resource limitation of larger cells. How did the light change between in-situ conditions and that in the incubator? What was the integrated PAR over the previous hours prior to the Fv/Fm measurement being made (if not at night time)? Perhaps different levels of PSII damage/down-regulation could be an explanation. Could the observed community shift not contribute to the Fv/Fm increases, i.e. do larger cells not typically have higher Fv/Fm (Suggett et al. 2009)? Should any chance of Fe contamination in the control bottles (e.g. during the 50L carboy) be acknowledged, as this would also be consistent with the observed responses?

The most likely cause of this rapid change in Fv/Fm in both treatments in Experiment 1, is as the reviewer correctly suggests a result of the change in the light environment between in situ and incubator conditions. The average daily PAR for the incubators was 6.52 - 6.99 mol photons m$^{-2}$ d$^{-1}$, which was a 3% increase for experiment 2 and a 16% decrease for experiment 3, in comparison to a 62% decrease for experiment 1 when compared to average daily in situ PAR.

Contamination of the 50 L carboy is unlikely to be a source of contamination as this would have propagated to the control treatments of experiments 2 and 3.

The observed community shift could explain the increase as larger cells do tend to have a higher Fv/Fm and $\sigma_{PSII}$, but without taxonomic data at 72 h when this change occurs it is impossible to determine whether this response is physiological, taxonomic or a combination.

The following text has been added to the discussion in an attempt to adequately address all of the above:

Line 330: “The total daily PAR in the incubators ranged from 6.52 - 6.99 mol photons m$^{-2}$ d$^{-1}$, which is in good agreement for the in situ light environments of experiments 2 and 3. However, this was a ~62% decrease in the daily PAR that the phytoplankton community in experiment 1 were previously subjected to. Such a decrease in PAR would be expected to lead to a decrease in the downregulation of PSII by photodamage, coincident with an anticipated response in community structure. This could explain the observed increase in Fv/Fm and decrease in $\sigma_{PSII}$, as larger cells tend to have a higher Fv/Fm and small $\sigma_{PSII}$ in comparison to smaller cells (Suggett et al., 2009). Indeed we did observe a change in the community structure for experiment 1 (Fig. S2),
suggestive that a decrease in light pressure resulted in a community response in the control treatment. However, the lack of taxonomic data at 72 h makes it difficult to distinguish whether the primary driver of this response is physiological, taxonomic or a combination of both.”

References to seasonal Fe supply (e.g. lines 315-318, 341 onwards): Reduced Fe concentrations through the growing season are not actually observed. Furthermore, Fv/Fm values are low at both the beginning and end of the growing season. The only data the authors have to go on is the more pronounced phytoplankton responses to Fe relative to controls later in the growing season. Please rephrase these sections to more clearly indicate specifically what your bioassay results can actually say about seasonal changes in Fe supply to mixed layer waters.

Depth-resolved nutrient concentrations have been added to table 1 and discussed in the results.

Line 239: “Mean silicate concentrations in the mixed layer were considered limiting and decreased between experiments (1.49 - 0.84 μM), mean phosphate and DFe also displayed a gradual seasonal depletion (0.77 - 0.65 μM and 0.22 - 0.09 nM respectively); whereas mean nitrate concentrations increased throughout the growing season (10.41 - 12.92 μM) (Table 1).”

However, we are in agreement with the reviewer that the conclusion made based upon the data presented here are too strong. As such, specific references to iron limitation now refer to their specific effects upon maintaining potential maximal growth and productivity.

Lines 311-312: ‘large diatoms would require an increased silicate concentration, which is a limiting macronutrient in this region’. Is silicate a limiting nutrient at your site? Concentrations over 1μM were measured and chlorophyll-a and diatoms were enhanced in all +Fe treatments without added silicate.

Silica limitation is a well known effect in the sub-Antarctic zone as discussed in previous studies (see Hutchins et al., 2001 & Boyd et al. 2010). Furthermore, as I have discussed the silica requirements for small diatoms is much less than that of large diatoms. So without a more in depth analysis of the community structure, i.e. microscopy samples, it is impossible to determine whether this shift seen in the treatments to more diatom dominated is large or small cells.

To clarify this further, the following statement was added to the text.

Line 345: “The addition of iron also resulted in changes at the community level switching from haptophyte to diatom dominated communities (Fig. S2) despite apparent silica limitation (1.49 - 0.84 μM), typical of the region (Hutchins et al., 2001; Boyd et al. 2010). This suggests a switch to smaller diatoms, which have lower silica requirements than larger ones (Hutchins et al., 2001), however without microscopy it is not possible to say for sure.”
The authors state mean chlorophyll over the euphotic zone was higher than that over that of the mixed layer and then interpret this as a result of insufficient iron within the mixed layer. Whilst this is a possibility, could accumulation of lower light acclimated (higher chlorophyll/cell) phytoplankton below the mixed layer not equally play a role?

This is definitely a possibility that phytoplankton below the mixed layer but within the euphotic zone have increased their chlorophyll:carbon ratios, so an analysis was performed to look at the backscatter (another proxy for phytoplankton biomass) and we do see increased values within this sub-mixed layer zone. Whilst there is evidence of enhanced chlorophyll:carbon ratios, this sub-mixed layer population has higher values in both parameters when compared to the mixed layer.

Sections of chlorophyll and backscatter have been added to the supplementary information to show this effect more clearly, see supplementary figure 3.

Precaution must however be taken when investigating changes in Chl-a concentration, as a proxy for phytoplankton biomass (Behrenfeld et al., 2016; Bellacicco et al., 2016; Mignot et al., 2014; Westberry et al., 2008; Westberry et al., 2016), as the higher average concentration over the euphotic
zone (0.8 mg m\(^{-3}\)) relative to the shallower mixed layer (0.4 mg m\(^{-3}\)) may represent a Chl-a packaging effect due to lower light levels at depth. As such, concurrent particulate backscatter (\(b_{bp}\)) (Fig. S3b) was investigated as an alternate proxy for phytoplankton biomass (Loisel et al., 2002; Stramski et al., 1999), which similarly depicted the presence of a subsurface bloom in response to anticipated iron relief at depth."

Lines 374–378: The mechanism the authors describe to explain the lack of Fe stress despite low Fe concentrations is not clear. The authors state that the 'limiting' nutrient ‘would be expected to be severely depleted through biological uptake regardless of resupply'. But the authors state that they observe no iron limitation early in the season, which is at odds with this explanation. If Fe was not limiting it should either accumulate in the dissolved phase, be scavenged, or taken up by the phytoplankton and stored even though it is not liming. A re-phrase here might be necessary.

This phrase is referring to the principle idea that DFe concentrations do not make a good proxy for iron limitation because it does not take into account the bioavailability of iron. There are additional sources of iron that are not taken into account with this measure, i.e. ligands, particulates etc. (and in addition the rate of supply is not measured). The results from the full depth profile confirm that iron does not accumulate in the dissolved phase (see figure below taken from Mtshali et al. (In Prep)), mean DFe concentrations decrease across the growing season to minimum concentrations in February. The mean concentrations of DFe in the mixed layer have since been added to Table 1.

Figure: Mean concentrations of DFe (nM) in different depth bins during the four occupations of the SAZ. 0 – 200 m (winter reservoir defined by depth of
maximum MLD), 0 – 82 m (euphotic zone defined by mean 1% light depth), subsurface reservoir 82 - 200 m and the mixed layer reservoir (MLD = 190 m, 32 m, 55 m and 43 m in July, December, January and February, respectively).

Within the current data set we are unable to calculate how much DFe is lost through scavenging, but it is possible that a significant portion of iron within the surface layers may be lost to this process. If phytoplankton had taken up this iron and stored it internally for when it may become a limiting nutrient then we would not see the responses present in the experiments when provided additional iron.

To provide further clarity, the text has been amended to as follows. Line 376:

“The transition from no response in experiment 1 to an increased response in experiments 2 and 3 is indicative of an increase seasonal iron limitation, similar to that observed in the high latitude North Atlantic (Ryan-Keogh et al., 2013), where available iron is depleted early in the growing season and additional resupply is insufficient to meet biological demands during the latter parts of the growing season, driving characteristic HNLC conditions. A progressive decrease in ambient iron concentrations (mean in the mixed layer; Table 1) in the SAZ, are also suggestive of a seasonal progression of iron limitation, however worth bearing in mind is that nutrient concentrations are often a poor indicator of iron limitation, as any limiting nutrient would be expected to be severely depleted through biological uptake with resultant ambient concentrations that remain close to zero despite possible event scale supply (Ryan-Keogh et al., 2017a).”

Lines 383-384: ‘The short transient periods of increased wind stress thus appear to provide temporal relief from Fe stress’. Where is the data (wind, mixed layer depths, dFe concentrations, Fe stress status) to support this?

Despite having the wind, MLD and mean in the mixed layer DFe concentrations, we agree that the dataset cannot support such a bold statement, which has thus been removed. However, we do feel that the data supplied in context with the references (Little et al., In Review) are sufficient to support the importance of sub-seasonal storm events in surface mixed layer DFe supply (e.g. periods of low wind stress lead to very shallow and persistent mixed layers with proposed DFe limitation driving subsurface blooms). The text has since been modified, line 401:

“A SAZ glider study by Little et al. (In Review) corroborated these findings with summer matchups in small-scale temporal variability (< 10 days) in wind stress, MLD and chlorophyll that emphasizes the interconnectedness between physical drivers and their biological response. Despite the similarity in the scales of variability, no correlation was observed between MLD and Chl-a, which is explained by the variable response that MLD adjustments drive, i.e. dilution (a decrease in Chl-a with increasing MLD) and growth (an increase in Chl-a with increasing MLD in response to nutrient entrainment) (Fauchereau et al., 2011). Both of these scenarios can be observed in the glider time series from this study (Fig. 4), where increased wind stress and deeper MLDs were
associated with both reduced (15 – 29 December) and enhanced (29 January – 7 February) Chl-a. The mid- to late summer experiments were set up during periods of low wind stress (<0.2 N m⁻²) with shallow MLDs, which may corroborate the positive response to iron relief observed in experiments 2 and 3. Worth noting is that the time period between 10 January and 29 January is when the SAZ experienced uncharacteristically low winds (Braun, 2008) for an extended period of time, driving shallow MLDs (~20 m) and the development of subsurface Chl-a (Fig. S3a), indicative of iron limitation within the mixed layer and a supply mechanism (seasonal/sub-seasonal/remineralized or storm driven) that is not sufficient to meet mixed layer phytoplankton demands. Precaution must however be taken when investigating Chl-a concentration as a proxy for phytoplankton biomass (Behrenfeld et al., 2016; Bellacicco et al., 2016; Mignot et al., 2014; Westberry et al., 2008; Westberry et al., 2016), as a higher average concentration over the euphotic zone (0.8 mg m⁻³) relative to the shallower mixed layer (0.4 mg m⁻³) may represent a Chl-a packaging effect due to lower light levels at depth (rather than an increase in biomass). As such, particulate backscatter (bₚ) (Fig. S3b) was investigated as an alternate proxy for phytoplankton biomass (Loisel et al., 2002; Stramski et al., 1999), which similarly depicted the presence of a subsurface bloom in response to anticipated iron relief at depth.”

Lines 389: Increased nitrate concentrations throughout the growing season: do the authors need to invoke iron limitation as reducing the availability of photosynthetic reductant for nitrate reduction? The increase in nitrate concentration is large 8 uM: could physical process, such as a greater contribution of more recently upwelled water, be used to explain this? Or is the temperature-derived ML not capturing enhanced surface stratification later in the season that restricts downward mixing of more nitrate depleted surface-most waters down to the incubation water collection depth? To test this the authors could calculate the buoyancy frequency in addition to the mixed layer depth.

Please note that an analysis of replicate macronutrient samples (DIN + phosphate) were performed due to quality controls found within a concomitant study (Mtshali et al, In Prep) and therefore the initial conditions have been updated to reflect this. In particular note that there is still an increase in nitrate but this is not as extreme as 8 uM, the increase is now ~3 uM.

The buoyancy frequency was calculated and is presented in the figure below, with the experimental dates and depths plotted. Towards late summer there is the appearance of a secondary stratification layer but this is below the experimental depth. So mixing between the surface and the experimental depth is unrestricted during each of the occupations. However, the variability in the mixed layer and stratification layer could result in fluxes from below that could explain the increase in nitrate.
Line 427: “What is potentially hard to reconcile with sustained seasonal productivity and a seasonal decrease in phosphate, silicate, and DFe is the observed increase in nitrate. However, this too is suggestive of community level iron limitation, as iron limitation can reduce the availability of photosynthetic reductant for nitrate reduction which can lead to the excretion of excess nitrate back into the water column (Cochlan, 2008; Lucas et al., 2007; Milligan and Harrison, 2000; Moore et al., 2013; Price et al., 1994). This, together with the likely resupply of nitrate from below the mixed layer via sub-seasonal storm events, which is not accessible to phytoplankton uptake due to iron limitation of nitrate reductase, could account for the observed seasonal increase in mixed layer nitrate.”

Line 406-407: In the high latitude of North Atlantic and potentially North Pacific the cryosphere is important to seasonal dynamics? (ice/ground melting leading to enhanced stratification etc.)

The cryosphere is important in these other high latitude regions, so the sentence has now been amended to the following:

Line 455: “The biogeochemical significance of the Southern Ocean, including the highly productive Atlantic sector, will increase with respect to climate change (Marinov et al., 2006); particularly as the Southern Ocean is a HNLC region where the cryosphere is critical to seasonal dynamics (Massom and Stammerjohn, 2010).”

**Anonymous Referee #2**

General comments: In this paper by Ryan-Keogh et al the authors present data on nutrient (iron) addition bioassay style experiments conducted in the sub-Antarctic zone of the S. Ocean. The papers describes the varying response to iron–addition on the phytoplankton community over the growing season and characterises changes in physiology, nutrient uptake and community composition. The paper then discusses potential causes of the relationship between biological demand for Fe and supply. The paper is well presented and is a useful addition to the important understand of the controls and limitations on primary production in this important
oceanographic region. My main concern is that the authors are too strong in their conclusions (especially relating to the seasonal cycle) from a limited dataset and sections of the paper should better reflect these limitations of the study.

**Specific comments:**

Three incubation experiments have been conducted. Which this is valuable data it is still only three data points throughout the growing season. As such conclusions as to how this data relates to a seasonal cycle should be stated with a bit more consideration. Especially as often the authors claim a development in iron stress over the growing season while the most iron-stressed community seems to be mid-season? While this could be due to the selection of for example cells with reduced iron-requirements as iron-limitation develops it needs more openly discussed.

The most iron stressed community is mid-season, if examining the photophysiology alone, however experiment 3 displayed the greatest increases in growth rates following iron addition. A statement to this effect has been added to the discussion.

Line 412: “When examining the photophysiology alone, experiment 2 displayed the greatest response to iron addition (Fig. 2c) with significant responses also observed in Chl-a derived net growth rates (Fig. S1) and nitrate drawdown rates (Table 2). Experiment 3 displayed the greatest increases in growth rates following Fe addition (Fig. S1), while significantly higher Fᵥ/Fₘ was similarly observed (Fig. 2e).”

We agree with reviewer 2, who similarly raised concerns like reviewer 1, that more consideration should be taken in regards to the conclusions. As such, the text has been modified to discuss the implications of iron limitation here on the potential maximal growth rates and productivity.

Some examples of how the conclusions have been adjusted are provided below.

Line 25: “Here we demonstrate that at the beginning of the growing season, there is sufficient iron to meet the demands of the phytoplankton community, but that as the growing season develops the mean iron concentrations in the mixed layer decrease and are insufficient to meet biological demand.”

Line 434: “Irrespective of the different supply mechanisms; winter-entrainment, storm driven entrainment, diapycnal diffusion, photochemical reduction or microbial regeneration, the iron supply to the mixed layer is not sufficient for phytoplankton to reach maximum growth potential and completely drawdown all available macronutrients.”

The authors infer accumulation of detached chlorophyll-binding protein as a mechanisms for low Fv/Fm during iron stress. If this is the case why does Fv/Fm not reduce in the set-up conditions (table 1). This is potentially a change in community – although the authors suggest the community is pretty consistent. Can the authors
One potential reason for the $F_v/F_m$ not reducing during the set-up conditions is that the phytoplankton species (haplophyte dominated - see Fig. S2) are living under steady-state iron limited conditions during experiments 2 and 3, high values of $F_v/F_m$ have previously been observed under steady state iron limitation in culture (Parkhill et al. 2001; Price 2005). However, with the lack of sufficient community structure data it is hard to determine which signal is dominating the measurements presented here. As the experiments will display a physiological signal (i.e. nutrient stress), superimposed with a taxonomic signal (i.e. different phytoplankton groups have different baseline $F_v/F_m$). It is also not possible to rule out the effects of light intensity on suppressing the initial $F_v/F_m$ measured, through the downregulation of PSII, when setting up the experiments. During the cruise, a CTD was deployed at 03:00 local time before sunrise, and a depth profile of FRRf was collected. Samples collected at 10m and 50m indicated a much higher $F_v/F_m$, with 0.32 and 0.39 respectively, in comparison to the initial samples collected 4 hours later after sunrise. Potential reasons for this discrepancy is that the dark acclimation step may have not fully relaxed the initial samples before measurement. Indeed, one of the results of the incubation was a ~62% decrease in the light exposure that the experimental bottles received, which potentially explains why both the controls and Fe increase their $F_v/F_m$ by ~0.15.

Depth profile of $F_v/F_m$ of the same station for samples that were collected on a CTD cast 4 hours prior to the experimental CTD cast.

Temperature was examined as a potential driver of $\Delta(F_v/F_m)$ during previous studies as part of my PhD, but was not found to be a significant driver in these studies and therefore excluded from the analysis (Ryan-Keogh et al., 2013; Ryan-Keogh et al., 2017). I have attached here 2 figures of data from these studies (unpublished), showing $\Delta(F_v/F_m)$ against temperature. However, given the small temperature range in the experimental set up, 10.44 - 10.8, it is
unlikely that there would be any temperature effect in dictating the range of $\Delta(F_v/F_m)$ values measured in the experiments.

HLNA results from Ryan-Keogh et al. 2013

Ross Sea results from Ryan-Keogh et al. 2017

Displayed here is the full time series of the changes in $F_m$ Chl$^{-1}$ and $F_v$ Chl$^{-1}$ from each experiment, evident in panels c & e is that the iron addition creates a difference in $F_m$ Chl$^{-1}$ between the treatments; there is no evidence of changes in $F_v$ Chl$^{-1}$ (panels d & f). However, I feel that the information in this figure is already displayed in figure 3c and 3d.
Technical corrections:

Line 27 – suggests depend is greater than supply – the supply rate could still be high

This sentence has now been modified to:

Line 31: “suggestive of seasonal iron depletion and an insufficient resupply of iron to meet biological demand.”

Line 74 – include a reference for this statement

The following reference has been added to the text on line 80 and included in the references.


Line 87 – define more what eddy-strom interactions mean

This section has now been updated to only discuss the effects of storm on shear mixing, rather than the effects of eddy-storm interactions on 3D mixing.
The complexities of these different mixing mechanisms is beyond the scope of this paper and is discussed in greater detail elsewhere. See line 93 for clarification.

Line 232 – Be clear if there was no sig difference throughout the experiment

There was no significant difference at any timepoint during the experiment, the text has been updated to state this more clearly.

Line 254: “Statistical analysis confirmed that there were no significant differences in F_v/F_m or chlorophyll throughout the experiment.”

Line 235 – I think the sigmaPSII data is in supplementary but please refer to this in the text

References to supplementary figure S1 which shows σ_{PSII} has been added to the text on lines 256, 264 and 272.

Figure 2 – I think the lines are mis-labelled - +Fe and control should be the other way around?

The labelling is correct on Figure 2, open circles which show the lowest values of F_v/F_m and chlorophyll are the controls. The closed circles represent the +Fe treatment which has the higher values of chlorophyll and F_v/F_m.

Line 304 – can you reference a paper that shows or discussed this bottle effect in more detail

Additional references have now been added - see line 327. This section discussing the bottle effects evident here have also been greatly expanded and discussed in detail.

“The rapid increase in F_v/F_m in both treatments from 24 h onwards is likely due to potential bottle effects i.e. a change in the light environment (Martin and Fitzwater, 1988; de Baar et al., 1990; Coale 1991; de Baar et al., 2005). The total daily PAR in the incubators ranged from 6.52 - 6.99 mol photons m^{-2} d^{-1}, which is in good agreement for the in situ light environments of experiments 2 and 3. However, this was a ~62% decrease in the daily PAR that the phytoplankton community in experiment 1 were previously subjected to. Such a decrease in PAR would be expected to lead to a decrease in the downregulation of PSII by photodamage, coincident with an anticipated response in community structure. This could explain the observed increase in F_v/F_m and decrease in σ_{PSII}, as larger cells tend to have a higher F_v/F_m and small σ_{PSII} in comparison to smaller cells (Suggett et al., 2009). Indeed, we did observe a change in the community structure for experiment 1 (Fig. S2), suggestive that a decrease in light pressure resulted in a community response in the control treatment. However, the lack of taxonomic data at 72 h makes it difficult to distinguish whether the primary driver of this response is physiological, taxonomic or a combination of both.”
We would like to thank you once again and hope that the changes made are sufficient to meet the requirements of publication in your journal.

Should you have any more comments or questions, then please do not hesitate to contact us.

Yours sincerely,

[Signature]

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Seasonal development of iron limitation in the sub-Antarctic zone

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Abstract

The seasonal and sub-seasonal dynamics of iron availability within the sub-Antarctic zone (SAZ, \textasciitilde 40 – 45\degree S) play an important role in the distribution, biomass and productivity of the phytoplankton community. The variability in iron availability is due to an interplay between winter entrainment, diapycnal diffusion, storm-driven entrainment, atmospheric deposition, iron scavenging and iron recycling processes. Biological observations utilising grow-out iron addition incubation experiments were performed at different stages of the seasonal cycle within the SAZ to determine whether supply mechanisms at the time of sampling were sufficient to meet biological demands at different times of the growing season. Here we demonstrate that at
the beginning of the growing season, there is sufficient iron to meet the demands of the phytoplankton community, but that as the growing season develops the supply mechanisms are insufficient to completely meet phytoplankton iron requirements. Phytoplankton increase their photosynthetic efficiency and net growth rates following iron addition from mid to late summer, with no differences determined during early summer; suggestive of seasonal iron depletion and an insufficient re-supply of iron to meet biological demand. The result of which is residual macronutrients at the end of the growing season, and the prevalence of the high-nutrient low-chlorophyll (HNLC) condition. We conclude that despite the prolonged growing season characteristic of the SAZ, which can extend into late summer/early autumn, results nonetheless suggest that iron supply mechanisms are insufficient to maintain potential maximal growth and productivity throughout the season.
1. Introduction

The Southern Ocean is an important region for atmospheric CO$_2$ drawdown, 30-40% of global anthropogenic carbon uptake (Khatiwala et al., 2009; Mikaloff Fletcher et al., 2006; Schlitzer, 2002), which is driven by phytoplankton community production and the biological carbon pump (BCP). The BCP is however sensitive to environmental influences that are associated with climate change, which include an intensification of the westerly winds (Le Quéré et al., 2009), and altered upwelling and mixed layer stratification (Bopp et al., 2005; Boyd, 2002). Together, these changes will impact the light and nutrient supply to the phytoplankton community, which could in turn alter the efficiency and extent of the BCP in the future.

The high productivity characteristic of this region is driven in part by the high macronutrient availability, while phytoplankton growth and productivity is ultimately constrained by the availability of light and iron (de Baar et al., 1990; Martin et al., 1990). The result of this limitation is the prevalence of macronutrients in the surface waters at the end of the growing season, resulting in the paradoxical high nutrient low chlorophyll (HNLC) conditions characteristic of the region. Further controls on the seasonal evolution and extent of the phytoplankton bloom include potential silicate limitation (Boyd et al., 2010; Hutchins et al., 2001), top-down controls by meso- and micro-zooplankton grazing (Dubischar and Bathmann, 1997; Moore et al., 2013; Pakhomov and Froneman, 2004; Smetacek et al., 2004) and seasonal/sub-seasonal changes in the critical and mixed layer depths (Fauchereau et al., 2011; Nelson and Smith, 1991).

Iron is a key component of photosynthesis due to the high requirements in the formation and function of key photosynthetic proteins, including photosystem I and photosystem II (Raven, 1990; Shi et al., 2007; Strzepek and Harrison, 2004). In addition, iron requirements by phytoplankton are closely linked to light availability, displaying an inverse relationship. Under
low light conditions phytoplankton can maximise photosynthesis in different ways; by either increasing the size or number of their photosynthetic units, the latter resulting in an increase in iron requirements under low light (Maldonado et al., 1999; Raven, 1990; Strzepek et al., 2012; Strzepek et al., 2011; Sunda and Huntsman, 1997). This close coupling of light and iron, that increases the cellular demand for iron under low light conditions can diminish light dependent photosynthesis when iron concentrations are too low to support growth (Hiscock et al., 2008; Moore et al., 2013; Ryan-Keogh et al., 2017b). Iron is also required in the function of both nitrate and nitrite reductase (de Baar et al., 2005), which function to facilitate the assimilation of nitrate and nitrite and their subsequent intracellular reduction to ammonium. In the Southern Ocean, and other HNLC areas, nitrate uptake rates are reported as becoming iron limited for this reason (Cochlan, 2008; Lucas et al., 2007; Moore et al., 2013; Price et al., 1994). However, rather than iron limitation directly inhibiting nitrate/nitrite reductase activity, the cause of reduced uptake rates may be the result of a bottleneck further downstream due to a lack of photosynthetically derived reductant (Milligan and Harrison, 2000). The result of this is excretion of excess nitrate and nitrite back into the water column, which combined with high rates of resupply relative to biological uptake, can culminate in HNLC conditions typical of the Southern Ocean.

The Atlantic sector of the Southern Ocean is composed of a series of water masses, each with distinct physical and chemical properties (Boyer et al., 2013), that are constrained by circumpolar fronts with large geostrophic velocities (Nowlin and Klinck, 1986; Orsi et al., 1995). The differing physical and chemical properties create a high degree of zonal variability within the biology, in particular the timing and extent of phytoplankton seasonal blooms (Thomalla et al., 2011). Key physical controls on this variability include sea ice cover and day length, yet this is not enough to explain the full range of measured variability. An alternative approach has examined whether the supply mechanisms of iron to the mixed layer differ
significantly in their extent allowing regions like the sub-Antarctic zone (SAZ) to exhibit prolonged summer blooms in comparison to the polar front zone (PFZ) (Thomalla et al., 2011). Tagliabue et al. (2014) postulated that due to weak diapycnal inputs of iron there must be a heavy reliance of Fe-recycling within the mixed layer to meet the iron demand. An alternative hypothesis is that summer storms can sustain mixed layer biomass through entrainment of limiting nutrients, particularly in the SAZ (Carranza and Gille, 2015; Nicholson et al., 2016; Swart et al., 2015). As a storm passes through the SAZ, it deepens the mixed layer accessing the subsurface iron reservoir, the subsequent re-shoaling of this buoyant water fuels surface water phytoplankton growth in a high light and replenished nutrient environment. The drivers of the seasonal characteristics of these regions is likely a combination of both factors, with variable dominance in time and space. Regardless, a greater understanding of the iron supply mechanisms and whether they meet the demand for phytoplankton growth over seasonal timescales is required.

This paper aims to test whether the phytoplankton community in the sub-Antarctic zone is seasonally limited by iron availability. This was done through a series of ship-board grow-out nutrient addition incubation experiments that were performed to determine the extent to which the addition of iron at different times of the growing season would relieve the phytoplankton from iron limitation driving changes in photophysiology, chlorophyll-a biomass and potential growth rates.

2. Materials and Methods

2.1. Oceanographic Sampling
The samples and data presented here were obtained during the annual Austral summer relief voyage of the South African National Antarctic Expedition 55 (SANAE 55) onboard the S.A. Agulhas II to the Atlantic sector of the Southern Ocean as part of the Southern Ocean Seasonal Cycle Experiment III (SOSCEx III, (Swart et al., 2012)); from the 3rd of December 2015 to the 11th of February 2016. During the cruise, 3 long-term (144 - 168 h) nutrient addition incubation experiments were performed within the sub-Antarctic zone of the Atlantic sector of the Southern Ocean (Fig. 1, Table 1) to determine whether relief from iron limitation drove variable changes in phytoplankton photophysiology and biomass over the growing season. Uncontaminated whole seawater was collected from 30 - 35 m depth in Teflon-lined, external closure 12 L Go-Flo samplers deployed on a trace metal clean CTD (Conductivity Temperature Depth) rosette system.

2.2. Nutrient addition incubation experiments

Nutrient addition incubation experiments were performed using methods similar to those employed previously in the Southern Ocean (Moore et al., 2007; Nielsdóttir et al., 2012; Ryan-Keogh et al., 2017a) and the high latitude North Atlantic (Ryan-Keogh et al., 2013). Water for experiments were transferred unscreened into an acid-washed 50 L LDPE carboy (Thermo scientific) to ensure homogenization; the homogenized water was then redistributed unscreened into 2.4 L polycarbonate bottles (Nalgene) for the experiments. The triplicate initial samples were collected from the same 50 L LDPE carboy. Experiments during the cruise were incubated under two treatments, control and iron addition (2.0 nM FeCl₃, ‘Fe’), at a constant screened (LEE filters) light level of 129.45 µmol quanta m⁻² s⁻¹. Light levels were determined using a handheld 4π PAR sensor (Biospherical Instruments), and set on a day:night cycle according to in situ sunset/sunrise times. Experiment incubations were conducted as biological
replicates with 16 bottles per treatment for each experiment, these were sub-sampled at set time points for key variables as outlined in the Supplementary Information (Table S1). Temperature was set at the in situ collection temperature for all samples. All bottle tops were externally sealed with film (Parafilm), and bottles were double bagged with clear polyethylene bags to minimize risk of contamination during the incubation. Subsampling of all experiments occurred at the same time of day as the initial set-up, see Table 1 for initiation times. All incubations were performed within customised Minus40 Specialised Refrigeration™ units, which were fitted with adjustable (intensity and timing) LED strips as well as a thermostat and cooling fan for temperature control.

2.3. Chlorophyll a and Nutrient Analysis

Samples for chlorophyll-a (Chl-a), 250 mL, were filtered onto GF/F filters and extracted into 90% acetone for 24 h in the dark at -20 °C, followed by analysis with a fluorometer (TD70; Turner Designs) (Welschmeyer, 1994). Macronutrient samples were drawn into 50 mL diluvials and stored at -20 °C until analysis on land. Nitrate + Nitrite and Silicate were measured using a Lachat Flow Injection Analyser (Egan, 2008; Wolters, 2002), whilst Nitrite and Phosphate were determined manually by colorimetric method as specified by Grasshoff et al. (1983). Dissolved iron samples (DFe) were filtered through 0.2 µm cartridge filters (Acropack) equipped with a 0.45 µm pre-filter, drawn into acid washed 125 mL LDPE bottles (Nalgene, Thermoscientific), acidified with 30% HCl suprapur to pH ~1.7 (using 2 mL L⁻¹ criteria), double bagged and stored at room temperature until analysis on land at the Université de Bretagne Occidentale (UBO), France using the Chemiluminescence – Flow Injection Analyser (CL-FIA) method (Obata et al., 1993; Sarthou et al., 2003). Accuracy and precision
of the method was verified by analysis of in-house internal standards and SAFe reference seawater samples (Johnson et al., 2007); the limits of detection were in the order of 10 pM.

2.4. Phytoplankton Photosynthetic Physiology

Variable chlorophyll fluorescence was measured using a Chelsea Scientific Instruments FastOcean fast repetition rate fluorometer (FRRf) integrated with a FastAct laboratory system. Samples were acclimated in dark bottles at in situ temperatures, and FRRf measurements were blank corrected using carefully prepared 0.2 µm filtrates for all samples (Cullen and Davis, 2003). FRRf measurements consisted of a single turnover (ST) protocol: 100 × 2 µs saturation flashlets with a 2 µs interval, followed by 25 × 1 µs relaxation flashlets with an interval of 84 µs, with a sequence interval of 100 ms. Sequences were repeated 32 times resulting in an acquisition length of 3.2 s. The power of the excitation LED (λ450) was adjusted between samples to saturate the observed fluorescence transients within a given range of $R_{\sigma PSII}$ (the probability of a reaction centre being closed during the first flashlet). $R_{\sigma PSII}$ was optimised between 0.042 to 0.064 as per manufacturer specifications. By adopting this approach, it ensures the best signal-to-noise ratio in the recovered parameters, whilst accommodating significant variations in the photophysiology of the phytoplankton community without having to adjust the protocol. Data from the FRRf were analysed to derive the fluorescence parameters as defined in Roháček (2002), by fitting transients to the model of Kolber et al. (1998) using the FastPro8 software (v1.0.55).

2.5. Phytoplankton Composition
Pigment samples from the incubation experiments were collected by filtering 0.5 – 2.0 L of water onto 25 mm GF/F filters. Filters were frozen and stored at -80 °C until analysis in Villefranche, France on a HPLC Agilent Technologies 1200. Filters were extracted in 100% methanol, disrupted by sonification, clarified by filtration and analysed by HPLC following the methods of Ras et al. (2008); limits of detection were on the order of 0.1 ng L^{-1}. Pigment composition data were standardized through root square transformation before cluster analysis utilizing multi-dimensional scaling, where similar samples appear together and dissimilar samples do not. Samples were grouped and analysed in CHEMTAX (Mackey et al., 1996) using the Southern Ocean specific pigment ratios from Gibberd et al. (2013). Multiple iterations of pigment ratios were used to reduce uncertainty in the taxonomic abundance as described in Gibberd et al. (2013), with the solution that had the smallest residual used for the estimated taxonomic abundance.

2.6. Ancillary physical data

Temperature and salinity profiles were obtained from a Sea-Bird CTD mounted on the rosette system. The mixed layer depth was calculated following de Boyer Montégut et al. (2004), where the temperature differs from the temperature at 10 m by more than 0.2°C ($\Delta T_{10m} = 0.2°C$). The position of the fronts were determined using sea surface height (SSH) data from maps of absolute dynamic topography (MADT) (Swart et al., 2010). The percentage euphotic depth was calculated as a function of the natural log of in situ photosynthetically active radiation (PAR) and the diffuse attenuation coefficient $K_d$. 

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2.7. Glider Dataset

Autonomous Seagliders (SG542 & SG543) were deployed in mooring mode in the sub-Antarctic zone of the Southern Ocean (43°S 8.5°E) as part of SOSCEx III. SG543 was deployed from 28 July 2015 to 8 December 2015, followed by SG542 which continued sampling until 8 February 2016. The deployment of both gliders resulted in a continuous high-resolution time series of 1832 profiles over 196 days, down to depths of 1000 m. The gliders measured a suite of parameters including conductivity, temperature, pressure, PAR, fluorescence and optical backscattering at two wavelengths (λ = 470 nm and 700 nm). At the deployment and retrieval of each glider cross-calibration CTD casts were performed (all within 3 km and 4 h of each other), yielding independent inter-calibrations between glider sensors and bottle samples of Chl-a. Glider fluorescence was corrected for quenching and converted to units of Chl-a (mg m⁻³), while glider backscattering was despiked, smoothed and converted to units of \( b_{bp} \) (m⁻¹), for specific details see Thomalla et al. (2017). Wind stress (N m⁻²) data was collected from a weather station mounted on a simultaneous deployment of a Liquid Robotics Wave Glider; wind stress was corrected to 10 m using the wind profile power-law (Irwin, 1967).

2.8. Data analysis

Sample means and standard deviations were calculated using Python, followed by tests for normality and equal variance prior to analysis of variance (ANOVA) to determine treatment effects (SciPy v0.17.1, Python v3.6). Significant results are reported at the 95% confidence level (\( p < 0.05 \)).
3. Results

The experiment set-up location in the SAZ spanned 66 days from the initiation of the first experiment to the initiation of the third experiment. Chlorophyll concentrations did not vary substantially between initiations, ranging from 0.84 – 0.97 mg m\(^{-3}\), alongside no significant variations in temperature or salinity (Table 1). Mean Silicate concentrations in the MLD were considered limiting and decreased between experiments (1.49 - 0.84 μM), mean phosphate and DFe also displayed a gradual seasonal depletion (0.77 - 0.65 μM and 0.22 - 0.09 nM respectively), whereas mean nitrate concentrations increased throughout the growing season (10.41 - 12.92 μM) (Table 1). Photophysiological measurements of quantum efficiency (F\(_{v}/F_{m}\)) ranged from 0.19 – 0.30 (with no seasonal trend) while a seasonal decrease in the cross-section of PSII (σ\(_{PSII}\)) was observed from 14.79 to 7.08 nm\(^{-2}\). All experiments were set up with water collected from above the mixed layer and the mean euphotic depth of 63.89±19.13 m, with the percentage of surface light ranging from 14.83 – 10.66 %.

Data from 144-168 h experiments in the SAZ indicated variable responses to iron addition to the extant phytoplankton community (Fig. 2). During ‘early-summer’ (experiment 1), no evidence for iron stress was observed as indicated in the similar responses in F\(_{v}/F_{m}\) (Fig. 2a) and chlorophyll (Fig. 2b) between iron addition (+ Fe) and control treatments; both variables increased to similar values at the end time point. Statistical analysis confirmed that there were no significant differences in F\(_{v}/F_{m}\) or chlorophyll throughout experiment 1. The effective cross-section of PSII (σ\(_{PSII}\) (nm\(^{-2}\)), Supplementary Information Fig. 1a) displayed a similar pattern with no significant differences between treatments, decreasing in both treatments to 5.68±0.27 and 5.63±0.13 for the control and iron addition treatments respectively. Experiment 2, initiated 28 days later in ‘mid-summer’, exhibited signs of iron limitation (Fig. 2c, 2d) with an increase in F\(_{v}/F_{m}\) from 0.30±0.02 to a maximum of 0.39±0.01 at 120 h in the + Fe treatment, whilst the
control ranged between 0.27 and 0.34 (Fig. 2c). Moreover, Chl-a concentrations were >2 times higher in the iron addition treatment compared to the control at the end time point (Fig. 2d). Significant differences were observed for Fv/Fm from 72 h onwards and for Chl-a concentrations from 120 h onwards. σPSII decreased to a minimum of 4.62±0.15 nm$^{-2}$ in the iron addition treatment at 120 h (Supplementary Information Fig. S1c), corresponding to the highest value in Fv/Fm; whereas the control treatment decreased from 6.45±0.23 to 5.96±0.13 nm$^{-2}$. The final experiment in ‘late-summer’ (experiment 3) displayed similar evidence for potential iron limitation within the extant phytoplankton community (Fig. 2e, f). Fv/Fm in the control treatment remained constant at 0.26±0.01, whereas in the iron addition treatment it increased to 0.33±0.01 (Fig. 2e). Chl-a concentrations were 2.5 times higher in the iron addition treatment compared to the controls after 144 h (Fig. 2f), resulting in significant differences in Fv/Fm from 24 h onwards. σPSII also decreased to a greater extent than the control in experiment 3 from 7.08±0.48 nm$^{-2}$ to a minimum of 5.45±0.15 nm$^{-2}$, compared to 6.23±0.14 nm$^{-2}$ in the control at 144 h (Supplementary Information Fig. S1e).

Chl-a specific growth rates ($\mu_{\text{Chl}}$) were calculated for each experiment (Table 2, Supplementary Information Fig. S1b, d, f), displaying significantly higher growth rates for the iron addition treatment in experiments 2 and 3 by up to 50% and 63% respectively, with no significant differences in experiment 1. Enhanced nitrate drawdown $\Delta(NO_3^-)$ was exhibited in experiment 2 (Table 2), with rates approximately 4 times higher than the other experiments. No enhanced drawdown of phosphate or silicate was exhibited in any of the experiments. Taxonomic abundance (Supplementary Information, Fig. S2), indicated that the dominant component of the community was Haptophytes (>40%) when all experiments were initiated. Experiment 1 displayed significant increases in Diatoms in both treatments, alongside a significant increase in Synechococcus in the control treatment. Experiments 2 and 3 displayed
similar results with significant increases in Diatoms but only following iron addition, with reductions in the Haptophyte group.

\( F_v/F_m \) is derived from measurements and analysis of the fluorescence kinetics of the photosynthetic reaction centre photosystem II (PSII) and associated light-harvesting antenna proteins (Kolber and Falkowski, 1993). Understanding the mechanistic changes in \( F_v/F_m \) can provide information on how the phytoplankton community respond to different stress factors. Increases in \( F_v/F_m \) following iron enrichment do not appear to be the result of an increase in PSII efficiency (\( F_v \)), but rather due to decreases in \( F_m \) and \( F_o \) (Behrenfeld et al., 2006; Lin et al., 2016; Macey et al., 2014; Ryan-Keogh et al., 2017a). To determine these relative changes in photophysiology, the absolute difference in \( F_v/F_m \) between the control and iron addition bottles was calculated at 24 h, \( \Delta(F_v/F_m) \) (Ryan-Keogh et al., 2013). \( \Delta(F_v/F_m) \) in experiment 1 was indistinguishable from zero (Fig. 3a), whereas in experiment 2 and 3 it was consistently positive with values of 0.08±0.01 and 0.06±0.00 respectively. These responses were markedly similar to the absolute differences in growth rates (Fig. 3b), with significantly higher differences in experiments 2 and 3. The absolute changes in maximum fluorescence (\( F_m \), Fig. 3c) and variable fluorescence (\( F_v \), Fig. 3d) normalized to chlorophyll were calculated to determine the mechanistic response. Significant differences were determined for \( F_m \) Chl\(^{-1} \) in experiments 2 and 3, with no significant differences in \( F_v \) Chl\(^{-1} \) across any experiments.

4. Discussion

Photosynthesis in the Southern Ocean is considered to be limited in winter by low mean irradiance, with net phytoplankton growth rates increasing rapidly following the onset of stratification in spring (Sverdrup, 1953). Despite these high levels of productivity and growth, complete macronutrient drawdown is not possible due primarily to constraints in the
availability of iron (Boyd et al., 2007; de Baar et al., 1990). Reasons for this growth limitation include the high iron requirements of the photosynthetic apparatus (Raven et al., 1999; Shi et al., 2007) particularly under low light conditions and a lack of iron sources (Duce and Tindale, 1991; Tagliabue et al., 2014). Phytoplankton blooms in the SAZ are characterized by high inter-annual and intra-seasonal variability with an extended duration that sustains high chlorophyll concentrations late into summer (Carranza and Gille, 2015; Racault et al., 2012; Swart et al., 2015; Thomalla et al., 2011; Thomalla et al., 2015). The longevity of these blooms is unusual as Fe limitation at this time of year is expected to be limiting growth (Boyd, 2002).

To determine the extent to which seasonal variability in the availability of iron is restricting phytoplankton photosynthesis and biomass accumulation in the SAZ, a series of grow-out nutrient addition incubation experiments were performed during the austral summer of 2015/2016.

The nutrient addition experiments (Fig. 2) demonstrated the development of seasonal iron limitation of the in situ phytoplankton population within the SAZ from early summer (December) to late summer (February). Experiment 1, which was set up during the early growing season did not display any significant differences between control and +Fe treatments (Fig. 2a, 2b). The rapid increase in Fv/Fm observed in both treatments from 24 h onwards is likely due to potential bottle effects in particular with respect to a change in the light environment (Martin and Fitzwater, 1988; de Baar et al., 1990; Coale 1991; de Baar et al., 2005). The total daily PAR in the incubators ranged from 6.52 - 6.99 mol photons m⁻² d⁻¹, which is in good agreement for the in situ light environments of experiments 2 and 3. However, this was a ~62% decrease in the daily PAR that the phytoplankton community in experiment 1 were previously subjected to. Such a PAR would be expected to lead to a decrease in the downregulation of PSII by photodamage, coincident with an anticipated response in community structure. This as larger cells tend to have a higher Fv/Fm and small σPSII in
comparison to smaller cells (Suggett et al., 2009). Indeed we did observe a change in the community structure for experiment 1 (Fig. S2), suggestive that a decrease in light pressure resulted in a community response. It makes it difficult to distinguish whether the primary driver of this response is physiological, taxonomic or a combination of both. When examining the photophysiology alone, eiron (Fig. 2c) with significant responses also observed in Chl-a derived net growth rates (Fig. S1) and nitrate drawdown rates (Table 2). Experiment 3 displayed the greatest increases in growth rates following Fe addition (Fig. S1), while was similarly observed (Fig. 2e). The addition of iron also resulted in changes at the community level switching from haptophyte to diatom dominated communities (Fig. S2) despite apparent silica limitation (1.49 - 0.84 μM), typical of the region (Hutchins et al., 2001; Boyd et al. 2010).

This suggests a switch to smaller diatoms, which have lower silica requirements than larger ones (Hutchins et al., 2001). Irregardless, this community shift is suggestive of community specific iron quota requirements (Ryan-Keogh et al., 2017a; Strzepek et al., 2012; Strzepek et al., 2011), which drive the composition of the extant phytoplankton community in the SAZ.

The increased responses with time are indicative of seasonal iron limitation, similar to the high latitude North Atlantic (Ryan-Keogh et al., 2013), where potential iron sources are depleted early in the growing season and there is insufficient iron resupply to meet biological demands during the growing season, driving characteristic HNLC conditions. Despite decreasing iron concentrations (mean in the MLD; Table 1) being suggestive of a seasonal progression of iron limitation, worth bearing in mind is that ambient concentrations are a poor indicator of iron limitation, as any limiting nutrient would be expected to be severely depleted through biological uptake with resultant ambient concentrations that remain close to zero despite possible event scale supply (Ryan-Keogh et al., 2017a).

Mechanistic changes in $F_{v}/F_{m}$, i.e. $\Delta(F_{v}/F_{m})$, are a useful proxy to determine the potential physiological signal of iron limitation without any superimposing taxonomic signal.
The derived variable $\Delta(F_v/F_m)$ was higher in experiments in 2 and 3 (Fig. 3a), with values consistent with studies from the North and South Atlantic and the Ross Sea (Browning et al., 2014; Ryan-Keogh et al., 2017a; Ryan-Keogh et al., 2013), which correlated well with the observed differences in net growth rates ($\Delta\mu_{\text{Chl}}$, Fig. 3b). Whilst no empirical relationship should be inferred between measures of photophysiology and measures of growth rates (Kruskopf and Flynn, 2006; Parkhill et al., 2001; Price, 2005), the observed correlation between these two independent variables suggests that a biomass independent measure of physiological iron stress, $F_v/F_m$, is likely accompanied by a significant repression of phytoplankton growth rates. These experiments also provide insight into the mechanistic iron-stress response of phytoplankton photophysiology, where increases in $F_v/F_m$ following iron addition are due to a reduction in the ratio of $F_m \text{Chl}^{-1}$ rather than $F_v \text{Chl}^{-1}$ (Fig. 3c, 3d). This is in agreement with similar observations made in the Ross Sea, the high latitude North Atlantic and equatorial Pacific (Behrenfeld et al., 2006; Lin et al., 2016; Macey et al., 2014; Ryan-Keogh et al., 2017a), all regions where the phytoplankton communities are subject to iron limitation. Elevated ratios of $F_m \text{Chl}^{-1}$ are potentially indicative of an energetically-decoupled pool of chlorophyll that possess a higher fluorescence yield than PSII at $F_m$ (Macey et al., 2014; Ryan-Keogh et al., 2017a; Ryan-Keogh et al., 2012; Schrader et al., 2011). These pools can be significant in iron limited regions with important implications for Chl-a derived primary productivity estimates that can be overestimated as a result (Behrenfeld et al., 2006; Macey et al., 2014).

The seasonal development of iron stress in the SAZ is suggestive of a primary dominant iron source to the surface waters, winter entrainment, which is subsequently depleted by upper ocean biota and abiotic scavenging onto settling particles (Tagliabue et al., 2014). Although diapycnal diffusion resupplies the mixed layer from late spring onwards, its low rates cannot be reconciled with potential phytoplankton uptake (Tagliabue et al., 2014). Instead, Tagliabue
et al. (2014) propose that biologically recycled iron within the mixed layer is the dominant mechanism for sustaining summertime blooms. However, there is now compelling evidence to suggest that storm events may also play a critical role in extending the duration of summertime production through intra-seasonal entrainment of dissolved iron from a subsurface reservoir (Carranza and Gille, 2015; Fauchereau et al., 2011; Swart et al., 2015; Thomalla et al., 2011). This mechanism was tested using a 1D biogeochemical model by Nicholson et al. (2016) whose results suggest that intra-seasonal mixed layer perturbations may offer relief from iron limitation in summer, particularly if there is sufficient subsurface vertical mixing beneath the surface mixed layer.

A SAZ glider study by Little et al. (in review) corroborated these findings with summer matchups Chl-aChl-aChl-aChl-amay corroborate that the time period between 10 January and 29 January is when (Braun, 2008) for an extended period of time, driving shallow MLD’s (~20 m) and the development of a subsurface Chl-a (Fig. S3a), indicative of iron limitation within the mixed layer and a supply mechanism (seasonal / sub-seasonal / remineralised or storm driven) that is not sufficient to meet mixed layer phytoplankton demands.

However be taken when investigating Chl-a y, the higher average concentration over the euphotic zone (0.8 mg m⁻³) relative to the shallower mixed layer (0.4 mg m⁻³) may represent a Chl-a As such, particulate backscatter (bₚ) (Fig. S3b) was investigated as an alternate proxy for phytoplankton biomass (Loisel et al., 2002; Stramski et al., 1999), which similarly depicted the presence of a subsurface bloom in response to anticipated iron relief at depth.

What is potentially hard to reconcile with sustained seasonal productivity and a seasonal decrease in phosphate, silicate nitratethis too is This, together with the likely resupply of nitrate from below the mixed layer via subseasonal storm events, which is not accessible to phytoplankton uptake due to Fe limitation of nitrate. Irrespective of the different supply mechanisms; winter-entrainment, storm driven entrainment, diapycnal
diffusion, photochemical reduction or microbial regeneration, the iron supply to the mixed layer over the summer season is not sufficient for phytoplankton to reach maximum growth potential and completely drawdown all available macronutrients. Moreover, this seasonal iron limitation may not be the only cause of sub-maximal productivity rates as silicate can also potentially limit phytoplankton growth in this region (Boyd et al., 2010; Hutchins et al., 2001). However, the significant shifts to diatom from haptophyte communities (Fig. S2) within the experimental treatments following iron addition suggest that silicate limitation may only be a secondary limiting factor.

The current study represents an analysis of the seasonal development of iron limitation in the SAZ, to shed light on whether iron supply mechanisms are sufficient to meet community demands. This is important for understanding iron (Massom and Stammerjohn, 2010) thus influence the overall extent of phytoplankton growth, macronutrient drawdown and ultimately the strength and efficiency of the biological carbon pump. However, the variations of supply in the seasonal cycle will also continue to play an important role in this ecological important oceanic region and warrant further investigation.

Acknowledgements

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Table 1: Locations of experiments conducted during the cruise along with details of the initial set up conditions.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
<th>Experiment 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>‘Early Summer’</td>
<td>‘Mid-Summer’</td>
<td>‘Late Summer’</td>
</tr>
<tr>
<td>Run time (h)</td>
<td>168</td>
<td>168</td>
<td>144</td>
</tr>
<tr>
<td>Initiation Date</td>
<td>08/12/2015</td>
<td>05/01/2016</td>
<td>08/02/2016</td>
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<tr>
<td>Initiation Time (GMT)</td>
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<td>20:00</td>
<td>02:00</td>
</tr>
<tr>
<td>Latitude (°S)</td>
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<td>-42.693</td>
<td>-43.000</td>
</tr>
<tr>
<td>Longitude (°E)</td>
<td>8.738</td>
<td>8.737</td>
<td>8.500</td>
</tr>
<tr>
<td>Collection Depth (m)</td>
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<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Sunrise:Sunset (GMT)</td>
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<td>04:00 – 19:00</td>
<td>04:40 – 18:40</td>
</tr>
<tr>
<td>Chl-a (mg m⁻³)</td>
<td>0.97</td>
<td>0.84</td>
<td>0.90</td>
</tr>
<tr>
<td>Nitrate (µM)</td>
<td>10.60</td>
<td>12.80</td>
<td>13.90</td>
</tr>
<tr>
<td>Mean in mixed layer</td>
<td>10.41±0.90</td>
<td>12.76±0.39</td>
<td>12.92±0.84</td>
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<tr>
<td>Silicate (µM)</td>
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<td>1.43</td>
<td>1.39</td>
</tr>
<tr>
<td>Mean in mixed layer</td>
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<td>1.41±0.02</td>
<td>0.84±0.13</td>
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<tr>
<td>Phosphate (µM)</td>
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<td>0.76</td>
<td>0.45</td>
</tr>
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<td>Mean in mixed layer</td>
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<td>0.76±0.06</td>
<td>0.65±0.21</td>
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<tr>
<td>DFe (nM)</td>
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<td>0.05</td>
</tr>
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<td>Mean in mixed layer</td>
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<td>0.15±0.003</td>
<td>0.09±0.01</td>
</tr>
<tr>
<td>Fv/Fm</td>
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<td>0.30±0.02</td>
<td>0.26±0.01</td>
</tr>
<tr>
<td>Experiment</td>
<td>$\mu_{\text{Chl}}$ (d$^{-1}$) 0 - end</td>
<td>$\Delta$(NO$_3^-$) (μmol L$^{-1}$ d$^{-1}$)</td>
<td></td>
</tr>
<tr>
<td>------------</td>
<td>---------------------------------</td>
<td>---------------------------------</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+ Fe</td>
<td>Control</td>
<td>+ Fe</td>
</tr>
<tr>
<td>1</td>
<td>0.28±0.02</td>
<td>0.27±0.02</td>
<td>0.98±0.005</td>
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<tr>
<td>2</td>
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<td>0.11±0.01</td>
<td>4.29±0.43</td>
</tr>
<tr>
<td>3</td>
<td>0.23±0.01</td>
<td>0.09±0.01</td>
<td>0.78±0.11</td>
</tr>
</tbody>
</table>

Table 2: Net growth rates calculated from Chl-a accumulation ($\mu_{\text{Chl}}$) and nitrate drawdown ($\Delta$(NO$_3^-$)) over the full experimental running time ($t = 168, 168, 144$ h).

Shown are averages with ± standard deviations, where $n = 6 - 12$ for Chl-a and $n = 6 - 7$ for nitrate (see Supplementary Information Table S1 for specific details).
Figure 1: Composite map of MODIS (8-day, 9 km) derived Chl-a (mg m$^{-3}$) from December 2015 to February 2016 for the Atlantic sector of the Southern Ocean, with locations of nutrient addition incubation experiments and the glider track. Inset composite map of absolute dynamic topography (MADT) from the CLS/AVISO product (Rio et al., 2011) from December 2015 to February 2016 with boundary definitions of sub-tropical front (STF) and sub-Antarctic front (SAF) (Swart et al., 2010), with locations of experiments and glider track.
Figure 2: Fv/Fm (a, c, e) and chlorophyll-a (Chl-a) responses (mg m⁻³) (b, d, f), from the control and Fe addition treatments of experiments initiated in the sub-Antarctic zone over early summer (a, b), mid-summer (c, d), and late summer (e, f). Displayed here are averages with ± standard deviations (n = 3 - 5 for all time points, except the end time point where n = 6 - 12, see supplementary information Table S1 for exact sample numbers). Please note the different scales in panels a and b.
Figure 3: (a) The difference in $F_v/F_m$ between the Fe treatment and control treatment ($\Delta(F_v/F_m)$) at the 24 h time point for experiments initiated in early summer (experiment 1), mid-summer (experiment 2) and late summer (experiment 3), where ($n = 3$ for $\Delta(F_v/F_m)$). (b) The difference in chlorophyll derived net growth rates ($\Delta\mu^{Chl} (d^{-1})$), where $t = 168, 168$ and 144 h. (c) The change in chlorophyll normalised maximum fluorescence, ($\Delta F_m^{Chl} (\text{Chl}^{-1})$). (d) The change in chlorophyll normalised variable fluorescence, ($\Delta F_v^{Chl} (\text{Chl}^{-1})$). Displayed here are averages with ± standard deviations ($n = 6$ for Experiment 1, 10 for Experiment 2, 12 for Experiment 3–5).
Figure 4: Time series from 6th December 2015 to 8th February 2016 of (a) surface wind stress (N m\(^{-2}\)), mixed layer depth (MLD, m) where \(\Delta T_{10m} = 0.2^\circ C\), and (b) mean chlorophyll-a concentration (mg m\(^{-3}\)) from the MLD and the euphotic zone. Experiment initiation dates are overlaid in grey bars.
Seasonal development of iron limitation in the sub-Antarctic zone

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Abstract

The seasonal and sub-seasonal dynamics of iron availability within the sub-Antarctic zone (SAZ, ~40 – 45°S) play an important role in the distribution, biomass and productivity of the phytoplankton community. The variability in iron availability is due to an interplay between winter entrainment, diapycnal diffusion, storm-driven entrainment, atmospheric deposition, iron scavenging and iron recycling processes. Biological observations utilising grow-out iron addition incubation experiments were performed at different stages of the seasonal cycle within the SAZ to determine whether supply mechanisms at the time of sampling were sufficient to meet biological demands at different times of the growing season. Here we demonstrate that at...
the beginning of the growing season, there is sufficient iron to meet the demands of the phytoplankton community, but that as the growing season develops the supply mechanisms are insufficient to completely meet phytoplankton iron requirements. Phytoplankton increase their photosynthetic efficiency and net growth rates following iron addition from mid to late summer, with no differences determined during early summer; suggestive of seasonal iron depletion and an insufficient re-supply of iron to meet biological demand. The result of which is residual macronutrients at the end of the growing season, and the prevalence of the high-nutrient low-chlorophyll (HNLC) condition. We conclude that despite the prolonged growing season characteristic of the SAZ, which can extend into late summer/early autumn, results nonetheless suggest that iron supply mechanisms are insufficient to maintain potential maximal growth and productivity throughout the season.
1. Introduction

The Southern Ocean is an important region for atmospheric CO\textsubscript{2} drawdown, 30-40% of global anthropogenic carbon uptake (Khatiwala et al., 2009; Mikaloff Fletcher et al., 2006; Schlitzer, 2002), which is driven by phytoplankton community production and the biological carbon pump (BCP). The BCP is however sensitive to environmental influences that are associated with climate change, which include an intensification of the westerly winds (Le Quéré et al., 2009), and altered upwelling and mixed layer stratification (Bopp et al., 2005; Boyd, 2002). Together, these changes will impact the light and nutrient supply to the phytoplankton community, which could in turn alter the efficiency and extent of the BCP in the future.

The high productivity characteristic of this region is driven in part by the high macronutrient availability, while phytoplankton growth and productivity is ultimately constrained by the availability of light and iron (de Baar et al., 1990; Martin et al., 1990). The result of this limitation is the prevalence of macronutrients in the surface waters at the end of the growing season, resulting in the paradoxical high nutrient low chlorophyll (HNLC) conditions characteristic of the region. Further controls on the seasonal evolution and extent of the phytoplankton bloom include potential silicate limitation (Boyd et al., 2010; Hutchins et al., 2001), top-down controls by meso- and micro-zooplankton grazing (Dubischar and Bathmann, 1997; Moore et al., 2013; Pakhomov and Froneman, 2004; Smetacek et al., 2004) and seasonal/sub-seasonal changes in the critical and mixed layer depths (Fauchereau et al., 2011; Nelson and Smith, 1991).

Iron is a key component of photosynthesis due to the high requirements in the formation and function of key photosynthetic proteins, including photosystem I and photosystem II (Raven, 1990; Shi et al., 2007; Strzepek and Harrison, 2004). In addition, iron requirements by phytoplankton are closely linked to light availability, displaying an inverse relationship. Under
low light conditions phytoplankton can maximise photosynthesis in different ways; by either increasing the size or number of their photosynthetic units, the latter resulting in an increase in iron requirements under low light (Maldonado et al., 1999; Raven, 1990; Strzepek et al., 2012; Strzepek et al., 2011; Sunda and Huntsman, 1997). This close coupling of light and iron, that increases the cellular demand for iron under low light conditions can diminish light dependent photosynthesis when iron concentrations are too low to support growth (Hiscock et al., 2008; Moore et al., 2013; Ryan-Keogh et al., 2017b). Iron is also required in the function of both nitrate and nitrite reductase (de Baar et al., 2005), which function to facilitate the assimilation of nitrate and nitrite and their subsequent intracellular reduction to ammonium. In the Southern Ocean, and other HNLC areas, nitrate uptake rates are reported as becoming iron limited for this reason (Cochlan, 2008; Lucas et al., 2007; Moore et al., 2013; Price et al., 1994). However, rather than iron limitation directly inhibiting nitrate/nitrite reductase activity, the cause of reduced uptake rates may be the result of a bottleneck further downstream due to a lack of photosynthetically derived reductant (Milligan and Harrison, 2000). The result of this is excretion of excess nitrate and nitrite back into the water column, which combined with high rates of resupply relative to biological uptake, can culminate in HNLC conditions typical of the Southern Ocean.

The Atlantic sector of the Southern Ocean is composed of a series of water masses, each with distinct physical and chemical properties (Boyer et al., 2013), that are constrained by circumpolar fronts with large geostrophic velocities (Nowlin and Klinck, 1986; Orsi et al., 1995). The differing physical and chemical properties create a high degree of zonal variability within the biology, in particular the timing and extent of phytoplankton seasonal blooms (Thomalla et al., 2011). Key physical controls on this variability include sea ice cover and day length, yet this is not enough to explain the full range of measured variability. An alternative approach has examined whether the supply mechanisms of iron to the mixed layer differ
significantly in their extent allowing regions like the sub-Antarctic zone (SAZ) to exhibit prolonged summer blooms in comparison to the polar front zone (PFZ) (Thomalla et al., 2011). Tagliabue et al. (2014) postulated that due to weak diapycnal inputs of iron there must be a heavy reliance of Fe-recycling within the mixed layer to meet the iron demand. An alternative hypothesis is that summer storms can sustain mixed layer biomass through entrainment of limiting nutrients, particularly in the SAZ (Carranza and Gille, 2015; Nicholson et al., 2016; Swart et al., 2015). As a storm passes through the SAZ, it deepens the mixed layer accessing the subsurface iron reservoir, the subsequent re-shoaling of this buoyant water fuels surface water phytoplankton growth in a high light and replenished nutrient environment. The drivers of the seasonal characteristics of these regions is likely a combination of both factors, with variable dominance in time and space. Regardless, a greater understanding of the iron supply mechanisms and whether they meet the demand for phytoplankton growth over seasonal timescales is required.

This paper aims to test whether the phytoplankton community in the sub-Antarctic zone is seasonally limited by iron availability. This was done through a series of ship-board growth-out nutrient addition incubation experiments that were performed to determine the extent to which the addition of iron at different times of the growing season would relieve the phytoplankton from iron limitation driving changes in photophysiology, chlorophyll-a biomass and potential growth rates.

2. Materials and Methods

2.1. Oceanographic Sampling
The samples and data presented here were obtained during the annual Austral summer relief voyage of the South African National Antarctic Expedition 55 (SANAE 55) onboard the S.A. Agulhas II to the Atlantic sector of the Southern Ocean as part of the Southern Ocean Seasonal Cycle Experiment III (SOSCEx III, (Swart et al., 2012)); from the 3rd of December 2015 to the 11th of February 2016. During the cruise, 3 long-term (144 - 168 h) nutrient addition incubation experiments were performed within the sub-Antarctic zone of the Atlantic sector of the Southern Ocean (Fig. 1, Table 1) to determine whether relief from iron limitation drove variable changes in phytoplankton photophysiology and biomass over the growing season.

Uncontaminated whole seawater was collected from 30 - 35 m depth in Teflon-lined, external closure 12 L Go-Flo samplers deployed on a trace metal clean CTD ( Conductivity Temperature Depth) rosette system.

2.2. Nutrient addition incubation experiments

Nutrient addition incubation experiments were performed using methods similar to those employed previously in the Southern Ocean (Moore et al., 2007; Nielsdóttir et al., 2012; Ryan-Keogh et al., 2017a) and the high latitude North Atlantic (Ryan-Keogh et al., 2013). Water for experiments were transferred unscreened into an acid-washed 50 L LDPE carboy (Thermo scientific) to ensure homogenization; the homogenized water was then redistributed unscreened into 2.4 L polycarbonate bottles (Nalgene) for the experiments. The triplicate initial samples were collected from the same 50 L LDPE carboy. Experiments during the cruise were incubated under two treatments, control and iron addition (2.0 nM FeCl3, ‘Fe’), at a constant screened (LEE filters) light level of 129.45 µmol quanta m⁻² s⁻¹. Light levels were determined using a handheld 4π PAR sensor (Biospherical Instruments), and set on a day:night cycle according to in situ sunset/sunrise times. Experiment incubations were conducted as biological...
replicates with 16 bottles per treatment for each experiment, these were sub-sampled at set time points for key variables as outlined in the Supplementary Information (Table S1). Temperature was set at the in situ collection temperature for all samples. All bottle tops were externally sealed with film (Parafilm), and bottles were double bagged with clear polyethylene bags to minimize risk of contamination during the incubation. Subsampling of all experiments occurred at the same time of day as the initial set-up, see Table 1 for initiation times. All incubations were performed within customised Minus40 Specialised Refrigeration™ units, which were fitted with adjustable (intensity and timing) LED strips as well as a thermostat and cooling fan for temperature control.

2.3. Chlorophyll a and Nutrient Analysis

Samples for chlorophyll-a (Chl-a), 250 mL, were filtered onto GF/F filters and extracted into 90% acetone for 24 h in the dark at -20 °C, followed by analysis with a fluorometer (TD70; Turner Designs) (Welshmeyer, 1994). Macronutrient samples were drawn into 50 mL diluvials and stored at -20 °C until analysis on land. Nitrate + Nitrite and Silicate were measured using a Lachat Flow Injection Analyser (Egan, 2008; Wolters, 2002), whilst Nitrite and Phosphate were determined manually by colorimetric method as specified by Grasshoff et al. (1983). Dissolved iron samples (DFe) were filtered through 0.2 µm cartridge filters (Acropack) equipped with a 0.45 µm pre-filter, drawn into acid washed 125 mL LDPE bottles (Nalgene, Thermoscientific), acidified with 30% HCl suprapur to pH ~1.7 (using 2 mL L⁻¹ criteria), double bagged and stored at room temperature until analysis on land at the Université de Bretagne Occidentale (UBO), France using the Chemiluminescence – Flow Injection Analyser (CL-FIA) method (Obata et al., 1993; Sarthou et al., 2003). Accuracy and precision...
of the method was verified by analysis of in-house internal standards and SAFe reference seawater samples (Johnson et al., 2007); the limits of detection were in the order of 10 pM.

2.4. Phytoplankton Photosynthetic Physiology

Variable chlorophyll fluorescence was measured using a Chelsea Scientific Instruments FastOcean fast repetition rate fluorometer (FRRf) integrated with a FastAct laboratory system. Samples were acclimated in dark bottles at in situ temperatures, and FRRf measurements were blank corrected using carefully prepared 0.2 µm filtrates for all samples (Cullen and Davis, 2003). FRRf measurements consisted of a single turnover (ST) protocol, 100 × 2 µs saturation flashlets with a 2 µs interval, followed by 25 × 1 µs relaxation flashlets with an interval of 84 µs, with a sequence interval of 100 ms. Sequences were repeated 32 times resulting in an acquisition length of 3.2 s. The power of the excitation LED (λ450) was adjusted between samples to saturate the observed fluorescence transients within a given range of $R_{\text{PSII}}$ (the probability of a reaction centre being closed during the first flashlet). $R_{\text{PSII}}$ was optimised between 0.042 to 0.064 as per manufacturer specifications. By adopting this approach, it ensures the best signal-to-noise ratio in the recovered parameters, whilst accommodating significant variations in the photophysiology of the phytoplankton community without having to adjust the protocol. Data from the FRRf were analysed to derive the fluorescence parameters as defined in Roháček (2002), by fitting transients to the model of Kolber et al. (1998) using the FastPro8 software (v1.0.55).

2.5. Phytoplankton Composition
Pigment samples from the incubation experiments were collected by filtering 0.5 – 2.0 L of water onto 25 mm GF/F filters. Filters were frozen and stored at -80°C until analysis in Villefranche, France on a HPLC Agilent Technologies 1200. Filters were extracted in 100% methanol, disrupted by sonification, clarified by filtration and analysed by HPLC following the methods of Ras et al. (2008); limits of detection were on the order of 0.1 ng L⁻¹. Pigment composition data were standardized through root square transformation before cluster analysis utilizing multi-dimensional scaling, where similar samples appear together and dissimilar samples do not. Samples were grouped and analysed in CHEMTAX (Mackey et al., 1996) using the Southern Ocean specific pigment ratios from Gibberd et al. (2013). Multiple iterations of pigment ratios were used to reduce uncertainty in the taxonomic abundance as described in Gibberd et al. (2013), with the solution that had the smallest residual used for the estimated taxonomic abundance.

2.6. Ancillary physical data

Temperature and salinity profiles were obtained from a Sea-Bird CTD mounted on the rosette system. The mixed layer depth was calculated following de Boyer Montégut et al. (2004), where the temperature differs from the temperature at 10 m by more than 0.2°C ($\Delta T_{10m} = 0.2°C$). The position of the fronts were determined using sea surface height (SSH) data from maps of absolute dynamic topography (MADT) (Swart et al., 2010). The percentage euphotic depth was calculated as a function of the natural log of in situ photosynthetically active radiation (PAR) and the diffuse attenuation coefficient $K_d$. 
2.7. Glider Dataset

Autonomous Seagliders (SG542 & SG543) were deployed in mooring mode in the sub-Antarctic zone of the Southern Ocean (43°S 8.5°E) as part of SOSCEx III. SG543 was deployed from 28 July 2015 to 8 December 2015, followed by SG542 which continued sampling until 8 February 2016. The deployment of both gliders resulted in a continuous high-resolution time series of 1832 profiles over 196 days, down to depths of 1000 m. The gliders measured a suite of parameters including conductivity, temperature, pressure, PAR, fluorescence and optical backscattering at two wavelengths ($\lambda = 470$ nm and 700 nm). At the deployment and retrieval of each glider cross-calibrations were performed (all within 3 km and 4 h of each other), yielding independent inter-calibrations between glider sensors and bottle samples of Chl-a. Glider fluorescence was corrected for quenching and converted to units of Chl-a (mg m$^{-3}$), while glider backscattering was despiked, smoothed and converted to units of b$_{bp}$ (m$^{-1}$), for specific details see Thomalla et al. (2017). Wind stress (N m$^{-2}$) data was collected from a weather station mounted on a simultaneous deployment of a Liquid Robotics Wave Glider; wind stress was corrected to 10 m using the wind profile power-law (Irwin, 1967).

2.8. Data analysis

Sample means and standard deviations were calculated using Python, followed by tests for normality and equal variance prior to analysis of variance (ANOVA) to determine treatment effects (SciPy v0.17.1, Python v3.6). Significant results are reported at the 95% confidence level ($p < 0.05$).
3. Results

The experiment set-up location in the SAZ spanned 66 days from the initiation of the first experiment to the initiation of the third experiment. Chlorophyll concentrations did not vary substantially between initiations, ranging from 0.84 – 0.97 mg m\(^{-3}\), alongside no significant variations in temperature or salinity (Table 1). Mean silicate concentrations in the MLD were considered limiting and decreased between experiments (1.49 – 0.84 µM), mean phosphate and DFe also displayed a gradual seasonal depletion (0.77 – 0.65 µM and 0.22 – 0.09 nM respectively), whereas mean nitrate concentrations increased throughout the growing season (10.41 – 12.92 µM) (Table 1). Photophysiological measurements of quantum efficiency (F\(_{v}/F\_m\)) ranged from 0.19 – 0.30 (with no seasonal trend) and a seasonal decrease in the cross-section of PSII (σ\(_{PSII}\)) was observed from 14.79 to 7.08 nm\(^2\). All experiments were set up with water collected from above the mixed layer and the mean euphotic depth of 63.89±19.13 m, with the percentage of surface light ranging from 14.83 – 10.66%.

Data from 144-168 h experiments in the SAZ indicated variable responses to iron addition to the extant phytoplankton community (Fig. 2). During ‘early-summer’ (experiment 1), no evidence for iron stress was observed as indicated in the similar responses in F\(_{v}/F\_m\) (Fig. 2a) and chlorophyll (Fig. 2b) between iron addition (+ Fe) and control treatments; both variables increased to similar values at the end time point. Statistical analysis confirmed that there were no significant differences in F\(_{v}/F\_m\) or chlorophyll throughout experiment 1. The effective cross-section of PSII (σ\(_{PSII}\) nm\(^{-2}\)) Supplementary Information Fig. 1a displayed a similar pattern with no significant differences between treatments, decreasing in both treatments to 5.68±0.27 and 5.63±0.13 for the control and iron addition treatments respectively. Experiment 2, initiated 28 days later in ‘mid-summer’, exhibited signs of iron limitation (Fig. 2c, 2d) with an increase in F\(_{v}/F\_m\) from 0.30±0.02 to a maximum of 0.39±0.01 at 120 h in the + Fe treatment, whilst the
control ranged between 0.27 and 0.34 (Fig. 2c). Moreover, Chl-a concentrations were 2 times higher in the iron addition treatment compared to the control at the end time point (Fig. 2d). Significant differences were observed for Fv/Fm from 72 h onwards and for Chl-a concentrations from 120 h onwards. σPSII decreased to a minimum of 4.62±0.15 nm\(^{-2}\) in the iron addition treatment at 120 h (Supplementary Information Fig. S1c), corresponding to the highest value in Fv/Fm whereas the control treatment decreased from 6.45±0.23 to 5.96±0.13 nm\(^{-2}\). The final experiment in ‘late-summer’ (experiment 3) displayed similar evidence for potential iron limitation within the extant phytoplankton community (Fig. 2e, f). Fv/Fm in the control treatment remained constant at 0.26±0.01, whereas in the iron addition treatment it increased to 0.33±0.01 (Fig. 2e). Chl-a concentrations were 2.5 times higher in the iron addition treatment compared to the controls after 144 h (Fig. 2f), resulting in significant differences in Fv/Fm from 24 h onwards. σPSII also decreased to a greater extent than the control in experiment 3 from 7.08±0.48 nm\(^{-2}\) to a minimum of 5.45±0.15 nm\(^{-2}\), compared to 6.23±0.14 nm\(^{-2}\) in the control at 144 h (Supplementary Information Fig. S1e).

Chl-a specific growth rates (µ\(^{\text{Chl}}\)) were calculated for each experiment (Table 2, Supplementary Information Fig. S1b, d, f), displaying significantly higher growth rates for the iron addition treatment in experiments 2 and 3 by up to 50% and 63% respectively, with no significant differences in experiment 1. Enhanced nitrate drawdown (\(\frac{\text{N}}{\text{t}}\)) was exhibited in experiment 2 (Table 2), with rates approximately 4 times higher than the other experiments. No enhanced drawdown of phosphate or silicate was exhibited in any of the experiments.

Taxonomic abundance (Supplementary Information, Fig. S2), indicated that the dominant component of the community was Haptophytes (>40%) when all experiments were initiated. Experiment 1 displayed significant increases in Diatoms in both treatments, alongside a significant increase in Synechococcus in the control treatment. Experiments 2 and 3 displayed...
similar results with significant increases in Diatoms but only following iron addition, with reductions in the Haptophyte group.

Fv/Fm is derived from measurements and analysis of the fluorescence kinetics of the photosynthetic reaction centre photosystem II (PSII) and associated light-harvesting antenna proteins (Kolber and Falkowski, 1993). Understanding the mechanistic changes in Fv/Fm can provide information on how the phytoplankton community respond to different stress factors. Increases in Fv/Fm following iron enrichment do not appear to be the result of an increase in PSII efficiency (Fv), but rather due to decreases in Fm and Fo (Behrenfeld et al., 2006; Lin et al., 2016; Macey et al., 2014; Ryan-Keogh et al., 2017a). To determine these relative changes in photophysiology, the absolute difference in Fv/Fm between the control and iron addition bottles was calculated at 24 h, Δ(Fv/Fm) (Ryan-Keogh et al., 2013). Δ(Fv/Fm) in experiment 1 was indistinguishable from zero (Fig. 3a), whereas in experiment 2 and 3 it was consistently positive with values of 0.08±0.01 and 0.06±0.00 respectively. These responses were markedly similar to the absolute differences in growth rates (Fig. 3b), with significantly higher differences in experiments 2 and 3. The absolute changes in maximum fluorescence (Fm, Fig. 3c) and variable fluorescence (Fv, Fig. 3d) normalized to chlorophyll were calculated to determine the mechanistic response. Significant differences were determined for Fm, Chl⁻¹ in experiments 2 and 3, with no significant differences in Fv, Chl⁻¹ across any experiments.

4. Discussion

Photosynthesis in the Southern Ocean is considered to be limited in winter by low mean irradiance, with net phytoplankton growth rates increasing rapidly following the onset of stratification in spring (Sverdrup, 1953). Despite these high levels of productivity and growth, complete macronutrient drawdown is not possible due primarily to constraints in the
availability of iron (Boyd et al., 2007; de Baar et al., 1990). Reasons for this growth limitation include the high iron requirements of the photosynthetic apparatus (Raven et al., 1999; Shi et al., 2007) particularly under low light conditions and a lack of iron sources (Duce and Tindale, 1991; Tagliabue et al., 2014). Phytoplankton blooms in the SAZ are characterized by high inter-annual and intra-seasonal variability with an extended duration that sustains high chlorophyll concentrations late into summer (Carranza and Gille, 2015; Racault et al., 2012; Swart et al., 2015; Thomalla et al., 2011; Thomalla et al., 2015). The longevity of these blooms is unusual as Fe limitation at this time of year is expected to be limiting growth (Boyd, 2002).

To determine the extent to which seasonal variability in the availability of iron is restricting phytoplankton photosynthesis and biomass accumulation in the SAZ, a series of grow-out nutrient addition incubation experiments were performed during the austral summer of 2015/2016.

The nutrient addition experiments (Fig. 2) demonstrated the development of seasonal iron limitation of the in situ phytoplankton population within the SAZ from early summer (December) to late summer (February). Experiment 1, which was set up during the early growing season did not display any significant differences between control and +Fe treatments (Fig. 2a, 2b). The rapid increase in $F_v/F_m$ observed in both treatments from 24 h onwards is likely due to potential bottle effects in particular with respect to a change in the light environment (Martin and Fitzwater, 1988; de Baar et al., 1990; Coale 1991; de Baar et al., 2005). The total daily PAR in the incubators ranged from 6.52 - 6.99 mol photons m$^{-2}$ d$^{-1}$, which is in good agreement for the in situ light environments of experiments 2 and 3. However, this was a ~62% decrease in the daily PAR that the phytoplankton community in experiment 1 were previously subjected to. Such a PAR would be expected to lead to a decrease in the downregulation of PSII by photodamage, coincident with an anticipated response in community structure. This as larger cells tend to have a higher $F_v/F_m$ and small $\sigma_{PSII}$ in...
comparison to smaller cells (Suggett et al., 2009). Indeed we did observe a change in the community structure for experiment 1 (Fig. S2), suggestive that a decrease in light pressure resulted in a community response, making it difficult to distinguish whether the primary driver of this response is physiological, taxonomic or a combination of both. When examining the photophysiology alone, e.g., $\Delta(F_v/F_m)$ with significant responses also observed in Chl-a derived net growth rates (Fig. S1) and nitrate drawdown rates (Table 2). Experiment 3 displayed the greatest increases in growth rates following Fe addition (Fig. S1), while was similarly observed (Fig. 2c). The addition of iron also resulted in changes to the community level, switching from haptophyte to diatom dominated communities (Fig. S2) despite apparent silica limitation (1.49 - 0.84 µM), typical of the region (Hutchins et al., 2001; Boyd et al. 2010). This suggests a switch to smaller diatoms, which have lower silica requirements than larger ones (Hutchins et al., 2001). Irregardless, this community shift is suggestive of community specific iron quota requirements (Ryan-Keogh et al., 2017a; Strzepek et al., 2012; Strzepek et al., 2011), which drive the composition of the extant phytoplankton community in the SAZ.

The increased responses with time are indicative of seasonal iron limitation, similar to the high latitude North Atlantic (Ryan-Keogh et al., 2013), where potential iron sources are depleted early in the growing season and there is insufficient iron resupply to meet biological demands during the growing season, driving characteristic HNLC conditions. Despite decreasing iron concentrations (mean in the MLD; Table 1) being suggestive of a seasonal progression of iron limitation, worth bearing in mind is that ambient concentrations are a poor indicator of iron limitation, as any limiting nutrient would be expected to be severely depleted through biological uptake with resultant ambient concentrations that remain close to zero despite possible event scale supply (Ryan-Keogh et al., 2017a).

Mechanistic changes in $F_v/F_m$, i.e., $\Delta(F_v/F_m)$, are a useful proxy to determine the potential physiological signal of iron limitation without any superimposing taxonomic signal.
The derived variable $\Delta(F_v/F_m)$ was higher in experiments 2 and 3 (Fig. 3a), with values consistent with studies from the North and South Atlantic and the Ross Sea (Browning et al., 2014; Ryan-Keogh et al., 2017a; Ryan-Keogh et al., 2013), which correlated well with the observed differences in net growth rates ($\Delta\mu_{Chl}$, Fig. 3b). Whilst no empirical relationship should be inferred between measures of photophysiology and measures of growth rates (Kruskopf and Flynn, 2006; Parkhill et al., 2001; Price, 2005), the observed correlation between these two independent variables suggests that a biomass independent measure of physiological iron stress, $F_v/F_m$, is likely accompanied by a significant repression of phytoplankton growth rates. These experiments also provide insight into the mechanistic iron-stress response of phytoplankton photophysiology, where increases in $F_v/F_m$ following iron addition are due to a reduction in the ratio of $F_m\ Chl^{-1}$ rather than $F_v\ Chl^{-1}$ (Fig. 3c, 3d). This is in agreement with similar observations made in the Ross Sea, the high latitude North Atlantic and equatorial Pacific (Behrenfeld et al., 2006; Lin et al., 2016; Macey et al., 2014; Ryan-Keogh et al., 2017a), all regions where the phytoplankton communities are subject to iron limitation. Elevated ratios of $F_m\ Chl^{-1}$ are potentially indicative of an energetically-decoupled pool of chlorophyll that possess a higher fluorescence yield than PSII at $F_m$ (Macey et al., 2014; Ryan-Keogh et al., 2017a; Ryan-Keogh et al., 2012; Schrader et al., 2011). These pools can be significant in iron limited regions with important implications for Chl-$a$ derived primary productivity estimates that can be overestimated as a result (Behrenfeld et al., 2006; Macey et al., 2014).

The seasonal development of iron stress in the SAZ is suggestive of a primary dominant iron source to the surface waters, winter entrainment, which is subsequently depleted by upper ocean biota and abiotic scavenging onto settling particles (Tagliabue et al., 2014). Although diapycnal diffusion resupplies the mixed layer from late spring onwards, its low rates cannot be reconciled with potential phytoplankton uptake (Tagliabue et al., 2014). Instead, Tagliabue...
et al. (2014) propose that biologically recycled iron within the mixed layer is the dominant mechanism for sustaining summertime blooms. However, there is now compelling evidence to suggest that storm events may also play a critical role in extending the duration of summertime production through intra-seasonal entrainment of dissolved iron from a subsurface reservoir (Carranza and Gille, 2015; Fauchereau et al., 2011; Swart et al., 2015; Thomalla et al., 2011). This mechanism was tested using a 1D biogeochemical model by Nicholson et al. (2016) whose results suggest that intra-seasonal mixed layer perturbations may offer relief from iron limitation in summer, particularly if there is sufficient subsurface vertical mixing beneath the surface mixed layer.

A SAZ glider study by Little et al. (in review) corroborated these findings with summer matchups Chl-a-Chl-c-Chl-p, corroborate that the time period between 10 January and 29 January is when (Braun, 2008) for an extended period of time, driving shallow MLD’s (∼20 m) and the development of a subsurface Chl-a (Fig. S3a), indicative of iron limitation within the mixed layer and a supply mechanism (seasonal / sub-seasonal / remineralised or storm driven) that is not sufficient to meet mixed layer phytoplankton demands.

However, be taken when investigating Chl-a, as the higher average concentration over the euphotic zone (0.8 mg m⁻³) relative to the shallower mixed layer (0.4 mg m⁻³) may represent a Chl-a. As such particulate backscatter (bₚₚ) (Fig. S3b) was investigated as an alternate proxy for phytoplankton biomass (Loisel et al., 2002; Stramski et al., 1999), which similarly depicted the presence of a subsurface bloom in response to anticipated iron relief at depth.

Limitation. What is potentially hard to reconcile with sustained seasonal productivity and a seasonal decrease in phosphate, silicate, nitrate is this too. This, together with the likely resupply of nitrate from below the mixed layer via subseasonal storm events, which is not accessible to phytoplankton uptake due to Fe limitation of nitrate recycling, irrespective of the different supply mechanisms; winter-entrainment, storm driven entrainment, diapycnal
diffusion, photochemical reduction or microbial regeneration, the iron supply to the mixed layer over the summer season is not sufficient for phytoplankton to reach maximum growth potential and completely drawdown all available macronutrients. Moreover, this seasonal iron limitation may not be the only cause of sub-maximal productivity rates as silicate can also potentially limit phytoplankton growth in this region (Boyd et al., 2010; Hutchins et al., 2001). However, the significant shifts to diatom from haptophyte communities (Fig. S2) within the experimental treatments following iron addition suggest that silicate limitation may only be a secondary limiting factor.

The current study represents an analysis of the seasonal development of iron limitation in the SAZ, to shed light on whether iron supply mechanisms are sufficient to meet community demands. This is important for understanding iron (Massom and Stammerjohn, 2010) influence the overall extent of phytoplankton growth, macronutrient drawdown and ultimately the strength and efficiency of the biological carbon pump. However, the variations of supply in the seasonal cycle will also continue to play an important role in this ecological important oceanic region and warrant further investigation.

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Table 1: Locations of experiments conducted during the cruise along with details of the initial set up conditions.

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<th>Experiment</th>
<th>'Early Summer'</th>
<th>'Mid-Summer'</th>
<th>'Late Summer'</th>
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<td>168</td>
<td>144</td>
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<tr>
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<td>04:00 – 19:00</td>
<td>04:40 – 18:40</td>
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<td>13.90</td>
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<td>Mean in mixed layer</td>
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<td>Phosphate (µM)</td>
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<tr>
<td>------------</td>
<td>---------------------</td>
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<td></td>
<td>± Fe</td>
<td>Control</td>
<td>± Fe</td>
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<td>3</td>
<td>0.23±0.01</td>
<td>0.09±0.01</td>
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</table>

Table 2: Net growth rates calculated from Chl-a accumulation (µ Chl) and nitrate drawdown (Δ(NO₃)) over the full experimental running time (t = 168, 168, 144 h).

Shown are averages with ± standard deviations, where n = 6 - 12 for Chl-a and n = 6 - 7 for nitrate (see Supplementary Information Table S1 for specific details).
Figure 1: Composite map of MODIS (8-day, 9 km) derived Chl-a (mg m⁻³) from December 2015 to February 2016 for the Atlantic sector of the Southern Ocean, with locations of nutrient addition incubation experiments and the glider track. Inset composite map of absolute dynamic topography (MADT) from the CLS/AVISO product (Rio et al., 2011) from December 2015 to February 2016 with boundary definitions of sub-tropical front (STF) and sub-Antarctic front (SAF) (Swart et al., 2010), with locations of experiments and glider track.
Figure 2: $F_v/F_m$ (a, c, e) and chlorophyll-a (Chl-a) responses (mg m$^{-3}$) (b, d, f), from the control and Fe addition treatments of experiments initiated in the sub-Antarctic zone over early summer (a, b), mid-summer (c, d), and late summer (e, f). Displayed here are averages with ± standard deviations ($n = 3 - 5$ for all time points, except the end time point where $n = 6 - 12$, see supplementary information Table S1 for exact sample numbers). Please note the different scales in panels a and b.
Figure 3: (a) The difference in $F_v/F_m$ between the Fe treatment and control treatment ($\Delta (F_v/F_m)$) at the 24 h time point for experiments initiated in early summer (experiment 1), mid-summer (experiment 2) and late summer (experiment 3), where ($n = 3$ for $\Delta (F_v/F_m)$). (b) The difference in chlorophyll derived net growth rates ($\Delta \mu_{Chl}^{t+1} \text{ (d}^{-1})$), where $t = 168, 168$ and 144 h. (c) The change in chlorophyll normalised maximum fluorescence, ($\Delta F_m/Chl$). (d) The change in chlorophyll normalised variable fluorescence, ($\Delta F_v/Chl$). Displayed here are averages with ± standard deviations ($n = 6$ for Experiment 1, 10 for Experiment 2, 12 for Experiment 3–5).
Figure 4: Time series from 6th December 2015 to 8th February 2016 of (a) surface wind stress (N m⁻²), mixed layer depth (MLD, m) where ΔT₁₀₀m = 0.2°C, and (b) mean chlorophyll-a concentration (mg m⁻³) from the MLD and the euphotic zone. Experiment initiation dates are overlaid in grey bars.