Temporal progression of photosynthetic-strategy in phytoplankton in the Ross Sea, Antarctica

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Abstract

The bioavailability of iron influences the distribution, biomass and productivity of phytoplankton in the Ross Sea, one of the most productive regions in the Southern Ocean. We mapped the spatial and temporal extent and severity of iron-limitation of the native phytoplankton assemblage using long- (>24 h) and short-term (24 h) iron-addition experiments along with physiological and molecular characterisations during a cruise to the Ross Sea in December-February 2012. Phytoplankton increased their photosynthetic efficiency in response to iron addition, suggesting proximal iron limitation throughout most of the Ross Sea during summer. Molecular and physiological data further indicate that as nitrate is removed from the surface ocean the phytoplankton community transitions to one displaying an iron-efficient photosynthetic strategy characterised by an increase in the size of photosystem II (PSII) photochemical cross section (σ_{PSII}) and a decrease in the chlorophyll-normalised PSII abundance. These results suggest that phytoplankton with the ability to reduce their photosynthetic iron requirements are selected as the growing season progresses, which may drive the well-documented progression from *Phaeocystis antarctica*- assemblages to diatom-dominated phytoplankton. Such a shift in the assemblage-level photosynthetic strategy potentially mediates further drawdown of nitrate following the development of iron deficient conditions in the Ross Sea.

Keywords

Iron, Phytoplankton, Photosynthetic proteins, Photosystem II, Nutrient limitation, Ross Sea

Highlights

- Phytoplankton in the Ross Sea change their photosynthetic physiology over the growing season to a strategy requiring less iron.
- This results in fluorescence yields per chlorophyll and PSII both increasing as the growing season develops.
- This observation may help explain the well characterised seasonal progression from to *Phaeocystis* spp. to diatom spp. over the growing season and also have implications for the assessment of primary production from estimates of chlorophyll in this region.

1. Introduction

The Ross Sea continental shelf is the most productive region in the Southern Ocean (Arrigo and van Dijken, 2004; Peloquin and Smith, 2007), with an annual productivity >200 g C m^{-2} (Smith et al., 2006), which may account for as much as 27% of the estimated total Southern Ocean biological CO_2 uptake (Arrigo et al., 2008). An understanding of the controls on primary productivity is therefore needed given the potential for future changes in stratification (Boyd et al., 2008; Smith et al., 2014) and nutrient inputs to this region (Mahowald and Luo, 2003; Tagliabue et al., 2008).

A persistent polynya in the southern Ross Sea greatly increases in size in the early austral spring (Arrigo and van Dijken, 2003; Reddy et al., 2007), and hosts large seasonal phytoplankton blooms, typically dominated by the colonial haptophyte *Phaeocystis antarctica* (*P. antarctica*) in spring through early summer (November – December), with an increase in abundance of diatoms in mid- to late summer (Arrigo and van Dijken, 2004; Arrigo et al., 1998; DiTullio and Smith, 1996; Goffart et al., 2000; Smith and Gordon, 1997; Smith et al., 2000). Understanding the causes and consequences of this seasonal phytoplankton progression is important, as the spatial and temporal distribution and abundance of *P. antarctica* and diatoms have significant biogeochemical consequences on, for example, the elemental composition and flux of biogenic material from the euphotic zone (Arrigo et al., 1999; DeMaster et al., 1992; Smith and Dunbar, 1998; Tagliabue and Arrigo, 2005).

Iron (Fe) and irradiance are assumed to exert the major 'bottom-up' controls on phytoplankton biogeography and productivity in the Ross Sea, given the incomplete macronutrient removal at the end of the growing season (Arrigo and van Dijken, 2003; Arrigo et al., 1998; Coale et al., 2003; Fitzwater et al., 2000; Sedwick et al., 2000; Sedwick et al., 2007; Smith et al., 2003; Smith et al., 2000; Tagliabue and Arrigo, 2003). Light availability may limit spring phytoplankton growth when vertical mixing is deep and daily integrated irradiance is low, this mixing will also supply dissolved iron (DFe) to the euphotic zone (McGillicuddy et al., 2015). As the growing season progresses and the water column stratifies, the flux of DFe from below is likely reduced and may therefore become a more significant factor in limiting

phytoplankton growth rates. Indeed, shipboard iron-addition experiments have repeatedly demonstrated the role of iron limitation in the Ross Sea (Bertrand et al., 2007; Coale et al., 2003; Cochlan et al., 2002; Martin et al., 1990; Olson et al., 2000; Sedwick and DiTullio, 1997; Sedwick et al., 2000), consistent with other metrics of Fe stress including high levels of flavodoxin (Maucher and DiTullio, 2003) and enhanced biological drawdown of silicate relative to nitrate (Arrigo et al., 2000; Smith et al., 2006).

Changes in phytoplankton composition from *P. antarctica* to diatom species may be linked to the co-limitation and interaction between iron and light. Boyd (2002) speculated that *P. antarctica* growth is limited by Fe availability from spring through late summer. Sedwick et al. (2007) further proposed that decreases in iron availability through spring are mitigated by increases in irradiance, thereby decreasing phytoplankton iron requirements. The differences in intracellular iron requirements alongside changes in the light environment may explain the community succession of the Ross Sea, where diatoms can outcompete *P. antarctica* in the late summer (Strzepek et al., 2012).

Phytoplankton that dominate in the Ross Sea may therefore need to be adapted to highly variable iron concentrations and light availability (Sedwick et al., 2011). An antagonistic relationship between irradiance and photosynthetic Fe demand may be predicted given that lower irradiances can increase Fe requirements associated with the synthesis of the additional photosynthetic units required to increase light absorption (Maldonado et al., 1999; Raven, 1990; Sunda and Huntsman, 1997). Each photosynthetic electron transfer chain requires 22-23 Fe atoms, and the photosynthetic apparatus can be the largest sink of Fe within a phytoplankton cell (Raven, 1990; Shi et al., 2007; Strzepek and Harrison, 2004). In contrast to the tight link between cellular Fe requirements and light harvesting capacity, studies on Southern Ocean diatoms and *P. antarctica* in culture suggest the Fe burden of photosynthesis may be significantly reduced for these species through increases in the size rather than the number of photosynthetic units (termed sigma-type acclimation) in response to iron/ and light limitation (Strzepek et al., 2012; Strzepek et al., 2011). Effectively, these Southern Ocean taxa appear to invest relatively more resources in the generation of a larger light-harvesting apparatus, rather than in the Fe-rich photosynthetic catalysts of photosystems I and II (Strzepek et al., 2012). This Fe-efficient strategy appears to be most pronounced for Southern Ocean diatoms, which, in culture can have some of the

largest light harvesting antennae reported (Strzepek et al., 2012), a phenotype which is more commonly associated with small cells (Suggett et al., 2009). The photosynthetic strategy of Southern Ocean diatoms may therefore contribute to the apparently low Fe requirement and cellular Fe:C ratio of these species (Coale et al., 2003; Kustka et al., 2015; Sedwick et al., 2007; Strzepek et al., 2012; Strzepek et al., 2011), and as such drive the seasonal progression from *P. antarctica* to diatoms in the Ross Sea.

In December-February 2012 a research cruise was conducted as part of the multidisciplinary research project *Processes Regulating Iron Supply at the Mesoscale – Ross Sea* (PRISM-RS), in an effort to identify and quantify the major sources of iron to the surface waters of the Ross Sea during the growing season. As part of this study, physiological and molecular measurements were combined with shipboard incubation experiments in an effort to define the spatial and temporal extent of phytoplankton iron limitation and reveal the photosynthetic strategy of the phytoplankton assemblages.

2. Materials and Methods

2.1. Oceanographic Sampling

The samples and data presented here were obtained during a cruise of the *RVIB Nathaniel B. Palmer* to the Ross Sea (cruise NBP12-01) from 24th December 2011 to 10th February 2012 (DOY 358 – 041). During the cruise, 29 short-term (24 h) and 3 long-term (168 h) incubation experiments were performed (Figure 1a). Short-term experiments were used to determine rapid iron induced changes in the phytoplankton photophysiological status; whereas long-term experiments determined whether relief from iron limitation could drive changes in biomass. For the long-term incubation experiments, uncontaminated whole seawater was collected from ~5 m depth whilst slowly underway, using a trace-metal clean towed fish system (Sedwick et al., 2011). Uncontaminated whole seawater for the short-term incubation experiments was collected from ~10 m depth in Teflon-lined, external closure 5 L Niskin-X samplers (General Oceanics) deployed on a trace metal clean CTD rosette system (Marsay et al., 2014). Samples for additional analysis were also collected along the cruise track.

2.2. Bioassay Incubation Experiments

Incubation experiments were performed using methods similar to those employed previously in the Southern Ocean (Moore et al., 2007; Nielsdóttir et al., 2012) and the high latitude North Atlantic (HLNA) (Nielsdóttir et al., 2009; Ryan-Keogh et al., 2013). Water for the experiments (see 2.1, above) was transferred unscreened into acid-washed 1.0-L polycarbonate bottles (Nalgene) for the short-term incubation experiments and 4.5-L polycarbonate bottles for the long-term incubation experiments. Incubation bottles were filled in a random order, with triplicate samples for initial measurements in the long-term incubation experiments collected at the beginning, middle and end of the filling process. Initial samples for the short-term incubation experiments were collected from the same Niskin-X sampling bottle. The short-term experiments were run for 24 h and the long-term experiments were run for 168 h; both experiments consisted of two treatments: an unamended control treatment

and 2.0 nmol L^{-1} Fe treatment (hereafter, + Fe). All experimental incubations were conducted as biological duplicates or triplicates.

All bottle tops were externally sealed with film (Parafilm™), and bottles were double bagged with clear polyethylene bags to minimize risks of contamination during the incubation. On-deck incubators were shaded using LEE "blue lagoon" filters to provide light levels corresponding to ~35% of above-surface irradiance (Hinz et al., 2012; Nielsdóttir et al., 2009; Ryan-Keogh et al., 2013). Flowing surface seawater was used to control the temperature in the incubators. Subsampling of long-term incubations for measurements of chlorophyll *a*, dissolved macronutrient concentrations and phytoplankton physiological parameters occurred after 24, 72, 120 and 168 h. Sub-sampling of short-term incubation experiments for the same parameters occurred after 24 h. All experiments were set up and sub-sampled under a class-100 laminar flow hood within a trace metal clean environment.

2.3. Chlorophyll *a* and Nutrient Analysis

Samples for chlorophyll *a* (Chl) analysis (250 mL) were filtered onto GF/F filters and then extracted into 90% acetone for 24 h in the dark at 4°C, followed by analysis with a fluorometer (TD70; Turner Designs) (Welschmeyer, 1994). Macronutrient samples were drawn into 50 mL diluvials and refrigerated at 4°C until analysis, which typically commenced within 12 h of sampling. Nitrate plus nitrite (DIN), phosphate, ammonium and silicate were determined shipboard on a five-channel Lachat Instruments QuikChem FIA+ 8000s series AutoAnalyser (Armstrong et al., 1967; Atlas et al., 1971; Bernhardt and Wilhelms, 1967; Patton, 1983). Dissolved iron was determined post-cruise using flow injection analysis modified from Measures et al. (1995), as described by Sedwick et al. (2011); accuracy of the DFe method was verified by analysis of SAFe reference seawater samples (Johnson et al., 2007).

2.4. Phytoplankton Photosynthetic Physiology

Variable chlorophyll fluorescence was measured using a Chelsea Scientific Instruments Fastracka™ Mk II Fast Repetition Rate fluorometer (FRRf) integrated with a FastAct™ Laboratory system. All samples were acclimated in opaque bottles for 30 minutes at *in situ* temperatures, and FRRf measurements were blank corrected

effect using carefully prepared 0.2 μm filtrates for all samples (Cullen and Davis, 2003). Blanks were typically around 1% and always <10% of the maximum fluorescence signal. Protocols for FRRf measurements and data processing were similar to those detailed elsewhere (Moore et al., 2007). Data from the FRRf were analysed to derive values of the minimum and maximum fluorescence (F_o and F_m) and hence F_v/F_m (where $F_v = F_m - F_o$), as well as the functional absorption cross-section of PSII (σ_{PSII}) by fitting transients to the model of Kolber et al. (1998).

2.5. Phytoplankton Composition

Samples for photosynthetic pigment analysis were collected and measured by high performance liquid chromatography (HPLC). 0.3 – 1.0 L of sea-water were filtered through GF/F filters, which were immediately flash frozen in liquid nitrogen and stored at -80°C until analysis. Pigments were extracted into 90% acetone by sonification before quantification using a Waters Spherisorb ODSU C-18 HPLC column and Waters HPLC system as described in Smith et al. (2006). Algal community composition was then estimated from pigment concentrations following the method of Arrigo et al. (1999).

2.6. Total Protein Extraction and Quantification

Photosynthetic protein abundances were quantified using techniques similar to those described elsewhere (Brown et al., 2008; Macey et al., 2014; Ryan-Keogh et al., 2012). Samples for protein extraction were collected by filtering 1.0-3.0 L of seawater onto GF/F filters (Whatman) under low light for ~45 minutes to minimize changes in protein abundance following sampling. Filters were flash frozen and stored at -80°C until analysis. Proteins were extracted in the laboratory according to the protocol described by Brown et al. (2008). Quantification was performed using custom Agrisera™ primary antibodies and peptide standards, which were designed against peptide tags conserved across all oxygenic photosynthetic species for protein subunits that are representative of the functional photosynthetic complex PsbA (PSII) (Campbell et al., 2003). Protein abundances were quantified using QuantityOne™ and ImageLab™ software; quantification was performed within the unsaturated portion of the calibration curve. The estimated protein abundances were comparable to those

reported for natural phytoplankton communities using similar methods (Hopkinson et al., 2010; Losh et al., 2013; Macey et al., 2014; Richier et al., 2012).

3. Results and Discussion

3.1. General Oceanography

A range of oceanographically distinct regions was occupied on the Ross Sea continental shelf during the PRISM-RS cruise (Figure 1). These included areas close to the Ross Ice Shelf, near and within pack ice, and over shallow bathymetric features, both of which may provide important sources of DFe to the upper water column (McGillicuddy et al., 2015). Highest chlorophyll *a* concentrations (Figure 2a) were associated with the ice-shelf in the southwestern Ross Sea (24.6 μg Chl L^{-1}) and correlated with the lowest DIN (dissolved inorganic nitrate + nitrite) concentrations (Figure 2b, Figure 3) and lowest surface F_v/F_m values observed (Figure 2c, Figure 3). Surface DFe concentrations ranged from 0.067-0.787 nM (Figure 2d), were not correlated with chlorophyll or DIN concentrations (Figure 3, Supplementary Information, Figure S1), and were elevated off the continental shelf in the northeast sector of the Ross Sea.

3.2. Mapping of Iron Limitation

Despite being the most productive region in the Southern Ocean, our results confirm that phytoplankton growth in the Ross Sea is limited by iron availability during summer, consistent with previous studies (Bertrand et al., 2011; Bertrand et al., 2007; Coale et al., 2003; Cochlan et al., 2002; Martin et al., 1990; Olson et al., 2000; Sedwick and DiTullio, 1997; Sedwick et al., 2000). The response of phytoplankton to iron-addition was assayed through a series of long- (168 h) and short-term (24 h) iron-addition incubations (Figure 1), while no clear spatial pattern in iron stress could be observed from a single cruise during a time of relatively rapid changes in a spatio-temporally complex system (Figure 2), there was evidence of an increase in photosynthetic efficiency following iron addition throughout much of the Ross Sea during summer, highlighting the role of iron in influencing phytoplankton physiology. To compare these iron-mediated changes in F_v/F_m, $\Delta(F_v/F_m)$ was calculated as defined in Ryan-Keogh et al. (2013), as the difference between the Fe-amended and control treatments (Equation 1).

279

280 Equation 1 Calculation of $\Delta(F_v/F_m)$.

$$\Delta\,(F_v/F_m) = \frac{F_v/F_{m_{+Fe}} - F_v/F_{m_{Control}}}{Time}$$

281 Values of $\Delta(F_v/F_m)$ were frequently positive following iron addition (ranging from
282 0.00 - 0.17) (Figure 4a), suggesting that Fe amendments increased the photosynthetic
283 efficiency of phytoplankton in much of the Ross Sea during the sampling period.

284 Data from long-term (168 h) experiments (Table 1 and Figure 4) enable a
285 more detailed analysis of the response of phytoplankton to iron-additions. Three
286 experiments were initiated from (1) near the Ross Ice Shelf, (2) over the Ross Bank
287 and (3) in an anti-cyclonic eddy (Figure 1 and Figure 4). The three experiments
288 revealed varying responses to iron additions by the extant phytoplankton assemblage.
289 Experiments 1 and 3 gave a strong and positive response to iron additions, and
290 provided evidence that phytoplankton were iron limited. Shorter-term responses
291 revealed elevated values of F_v/F_m (i.e., a positive $\Delta(F_v/F_m)$) after 24 h (Figure 4a),
292 with subsequent significant (ANOVA, $p<0.05$) increases in growth rates and nutrient
293 removal observed after 168 h (Table 1). Experiment 2, initiated over the Ross Bank,
294 did not show an increase in photosynthetic efficiency $\Delta(F_v/F_m)$ (Figure 4a).
295 Moreover, growth rate and nutrient removal were not significantly different between
296 control and iron-addition conditions until after >168 h (ANOVA, $p>0.05$) (Table 1),
297 which most likely reflects severe depletion of ambient DFe in the control treatments
298 by this time. The Ross Bank (Figure 4a, Table 1) has a shallow bathymetry (~150 m),
299 and none of the Fe-addition experiments in this region showed a significant response
300 (Figure 4). The Ross Bank may therefore provide significant and continuous DFe
301 inputs to the euphotic zone, thereby ultimately stimulating productivity.

302 The measurement of F_v/F_m is derived from analysis of the fluorescence kinetics
303 emitted from the photosynthetic reaction centre photosystem II (PSII) and its
304 associated light-harvesting antenna (Kolber and Falkowski, 1993). Understanding
305 the mechanism of changes to F_v/F_m can provide information on the process by which
306 phytoplankton respond to iron-limitation. Absolute changes in maximum fluorescence
307 (F_m) and variable fluorescence (F_v) normalised to chlorophyll *a* were calculated
308 (Figure 4b and 4c), revealing a significant difference between the +Fe and control
309 treatments in F_m Chl^{-1} (t = 24 h (*t*-test, $p<0.05$)), whereas there was no significant
310 difference for F_v Chl^{-1} (t = 24 h (*t*-test, $p>0.05$)). This suggests that changes in F_v/F_m

reflect changes in the proportion of chlorophyll that is photosynthetically coupled to active PSII reaction centres, rather than changes in the activity of PSII (Behrenfeld et al., 2006; Lin et al., 2016; Macey et al., 2014). A similar response was observed for all short-term iron-addition experiments that exhibited positive changes in $\Delta(F_v/F_m)$.

3.3 Temporal Development of Photosynthetic Strategy

Given the high degree of spatial variability in response to iron-additions, we placed all observations within a unified framework, hence producing a conceptualised model of temporal progression of phytoplankton within the Ross Sea. The PRISM-RS cruise sampled for 30 days covering a period from mid- to late summer, during which we expected iron limitation of phytoplankton growth to be significant (Sedwick et al., 2000). Total phytoplankton biomass accumulation is dependent on growth after the sampled regions become ice-free (Arrigo and van Dijken, 2003) and the losses due to grazing, sinking and physical removal. All spatial data therefore represent a mosaic of different temporal progressions that represent different stages of phytoplankton development. We utilise surface nitrate (DIN) as a proxy to separate the temporal patterns from any spatial differences (Figure 5). As phytoplankton biomass (Chl) increased, nutrients were removed and F_v/F_m reduced (Figure 3, Figure 5a). Pigment data showed that the nutrient drawdown and Chl increase in parallel with a shift from *P. antarctica*-dominated to diatom-dominated assemblages (Figure 3, Figure 5b). Within this conceptual framework, the relative severity of Fe-stress ($\Delta F_v/F_m$) may be inferred from the Fe-addition incubation experiments. Two potential phases of Fe deficiency were identified (Figure 5c): first, when DIN concentrations remain high (> ~20 µM) and *P. antarctica* is a major component of the phytoplankton (labelled '1'), and secondly when DIN is further removed (to < ~20 µM) by diatom-dominated communities (labelled '2'; Figure 5c).

Photophysiological parameters are presented within this framework. The relative size of the effective light-harvesting cross-section of PSII (σ_{PSII}) (Figure 6a) is low (~1.6 nm^{-2}) when DIN and F_v/F_m are high, and approximately doubles to ~3.29 nm^{-2} as DIN is depleted and the assemblage becomes diatom-dominated. Quantification of the photosynthetic catalyst PSII further characterises the photosynthetic strategy of phytoplankton in the Ross Sea. Chlorophyll normalised to abundances of the protein target PsbA (indicative of the abundance of PSII; (Brown et

al., 2008) (Chl:PsbA), which can provide another indication of the relative sizes of the light harvesting pigment antenna relative the abundance of the photosystems, is lower at higher DIN concentrations and increases as DIN and F_v/F_m decrease (Figure 6b). Combining the protein abundance data and the photophysiological measurements, the maximum fluorescent yield per chlorophyll (F_m:Chl) (Figure 6c) and per PSII (F_m:PsbA) (Figure 6d) can also be calculated. Both of these parameters increase, by 46 and 296% respectively, with decreases in DIN and F_v/F_m.

Together, these photophysiological measurements and corresponding environmental information at the time of sampling therefore indicate several significant correlations (Figure 3 & Supplementary Information, S1) associated with the potential drivers of the observed transition in community structure and subsequent changes in photophysiology. Thus, within our conceptual frame work, using DIN concentration as a proxy for the stage of the phytoplankton bloom, we observe statistically significant positive correlations (p<0.01) with other macronutrients and the photosynthetic efficiency (F_v/F_m) which all decline as nitrate is removed from the system. While negative correlations (p<0.01) are observed between DIN and temperature, chlorophyll concentration, the relative abundance of diatoms and σPSII which all increase as nitrate is removed from the system. While no significant correlation is seem between DIN and the fluorescence yield per PSII (F_m:PSII) or the chlorophyll content per PSII (Chl:PSII), there is a significant negative correlation between F_m:Chl and PSII:Chl (p<0.01) (Figure 3).

No statistically significant (p<0.01) relationships were observed with dissolved iron concentrations, suggesting that this variable may not represent a good indicator of iron stress, as might be expected considering that any limiting nutrient would be expected to be severely depleted by biological uptake. Overall, the observed correlations are thus taken to be indicative of the phytoplankton community transitioning between dominant groups as SST increases, non-limiting macronutrients are drawn down and the community biomass increases, potential as a result of different Fe utilisation capacities between diatoms and *P. antarctica* (Strzepek et al., 2012). These observations may also support that the hypothesis that Southern Ocean diatoms may both acquire (Kustka et al., 2015) and utilise (Strzepek et al., 2012) iron more effectively than *P. antarctica* and that the community transition may enable further drawdown of nitrate.

While there can be an array of reasons for diatoms being better at acquiring and utilising available DFe as it becomes limiting during summer in the Ross Sea, differences in photosynthetic strategy have the potential to be a significant factor in regulating the temporal changes that occur, given that the photosynthetic apparatus represents the dominant sink for Fe in a phytoplankton cell (Raven, 1990; Strzepek and Harrison, 2004). The analysis presented here clearly demonstrates that a different photosynthetic strategy is apparent within the phytoplankton community responsible for the initial DIN removal vs. those responsible for the later DIN removal. These observations of photosynthetic strategy are consistent with some of the ecophysiological differences observed within culture-based studies of Southern Ocean phytoplankton (Strzepek et al., 2012). Phytoplankton in the Ross Sea generally display a large, functional light-harvesting cross section for PSII (σ_{PSII}) compared to temperate species (Smith et al., 2011). As has been proposed (Strzepek et al., 2012), this may reflect a strategy by which cells acclimate and/or adapt through increasing the size of photosynthetic units rather than the number of photosynthetic units in a low Fe environment – thus escaping the typical antagonistic relationship between iron-demand and light capture (Sunda and Huntsman, 1997). Our measurements of the abundance of the photosynthetic catalysis PSII were also consistent with such an observation, whereby the increase in the ratio of Chl:PSII mirrors the increase in $\sigma_{PSII.}$ (Figure 6b). This strategy could significantly reduce the iron-demand normally associated with the photosynthetic apparatus. Phytoplankton that dominate at low DIN have a particularly large σ_{PSII} and have increased Chl:PSII values by 255%, again in agreement with culture studies in which Southern Ocean diatoms have larger σ_{PSII} than *P. antarctica* (Strzepek et al., 2012).

We thus suggest that the diatoms that dominate in summer as DIN is removed may represent a refined strategy to reduced iron availability, noting that previous information from temperate taxa and regions (Suggett et al., 2009) would tend to suggest that relatively high functional cross sections would be unlikely in phytoplankton with large cell sizes typical of many Southern Ocean diatoms (Suggett et al., 2009). Large cells with large σ_{PSII} may, however, result in ecophysiological trade-offs, including a tendency for over-excitation of PSII and photodamage, which may require a rapid PSII repair cycle or a requirement for rapidly inducible and significant non-photochemical quenching (Campbell and Tyystjärvi, 2012; Petrou et al., 2010; Wu et al., 2011), possibly suggesting Antarctic diatoms would require novel

412 photoprotective strategies. Despite these potential negative consequences of a large
413 σ_{PSII}, Antarctic diatoms seem to have adopted a phenotypic response underlining the
414 relevance of iron-availability and providing some explanation for the low Fe:C ratios
415 in some of these species (Strzepek et al., 2012).

416 While the observations in this study were restricted to the summer season they
417 do include DIN concentrations similar to those estimated for the winter mixed layer
418 nitrate concentration (McGillicuddy et al., 2015) and so potentially conditions
419 analogous to a broader seasonal progression in phytoplankton composition in the Ross
420 Sea from *P. antarctica* early in the growing season to diatom-dominance later in
421 summer (Smith et al., 2010). The dataset therefore provides indications of potential
422 contributory mechanisms for this seasonal progression, while also reflecting the large
423 degree of spatial heterogeneity in physical and biological processes throughout the
424 growing season in the Ross Sea (Smith and Jones, 2015).

425 The data presented here also provide insights into the mechanism of the iron-
426 stress response of phytoplankton. Increases in F_v/F_m are commonly reported as a
427 response to Fe addition (Boyd et al., 2008; Feng et al., 2010). Results from the
428 experiments and observations show that increases in F_v/F_m in response to Fe addition
429 and elevated F_v/F_m values in regions with modest DIN drawdown result from
430 reduction in the ratio of F_m:Chl (or F_m:PSII) rather than changes in F_v:Chl. This is in
431 agreement with similar observations from the high latitude North Atlantic and
432 Equatorial Pacific (Behrenfeld et al., 2006; Lin et al., 2016; Macey et al., 2014)
433 regions and implies that low F_v/F_m results from changes in the coupling of light-
434 harvesting chlorophyll-binding proteins to photosynthesis rather than accumulation of
435 damaged photosystems. Such accumulation of non-photosynthetically active
436 chlorophyll-binding proteins in Fe-limited oceanic regions can have consequences on
437 estimates of productivity in these regions (Behrenfeld et al., 2006).

4. Conclusions

The current study represents an analysis of the summer photosynthetic strategies of phytoplankton in the Ross Sea and highlights how different iron-efficiency strategies occur in phytoplankton as Fe becomes limiting and irradiance availability becomes maximal. This is important for understanding Fe usage efficiency in the region. The Ross Sea clearly differs from other high latitude regions due to plankton composition, yet iron availability still contributes to reduced growth rates and macronutrient removal. Even though this system is one of the most productive regions in the Southern Ocean, iron availability still exerts a strong control over summer productivity and biomass accumulation, and any changes in future iron supply induced by climate change could have profound effects. Climate-mediated changes to the mixed layer depth and sea-ice cover could change iron limitation strategies and phytoplankton phenology (Boyd et al., 2012), as well as alterations to iron supply from highly variable supply mechanisms such as Australian and local dust inputs (Mackie et al., 2008). The Southern Ocean is predicted to be particularly biogeochemically significant with respect to climate change (Marinov et al., 2006) and is the only iron-limited HNLC region where the cryosphere plays a major role. An understanding of the role of iron limitation in this highly dynamic environment is thus particularly important; particularly as climate mediated variability is expected to increase.

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Tables and Figure Legends

Table 1 Locations for long-term experiments conducted during NBP12-01 along with values of initial F_v/F_m, $\Delta(F_v/F_m)$ (Equation 1), net growth rates estimated from chlorophyll accumulation (Supplementary Information, Equation S1) and nitrate drawdown (Supplementary Information, Equation S2) over 168 h. Shown are averages ± standard errors (n = 3 or 5), * indicate significant differences (Two-way ANOVA, $p<0.05$) from control.

Figure 1 Composite map of Southern Ocean MODIS chlorophyll *a* for December 2011 – February 2012. Inset: Long-term (blue dots) and short-term (red dots) experimental locations conducted on cruise NBP12-01 in the Ross Sea with 250 m bathymetric contours. Surface *in situ* samples were also collected at these locations and at those marked CTD-station (black dots).

Figure 2 Surface chlorophyll concentrations (μg L^{-1}) from CTD stations **(a)**. Surface DIN concentrations (μM) **(b)**. Surface F_v/F_m **(c)**. Surface DFe concentrations (nM) **(d)**. Chlorophyll, DIN and F_v/F_m from samples collected at 1-5 m depth, DFe from samples collected at ~10 m depth.

Figure 3 Matrix of Pearson's linear correlation coefficients between the variables measured in the surface waters of the Ross Sea, including: sea surface temperature (SST), Nitrate (DIN), Phosphate (PO_4^{3-}), Silicate (Si), community structure (% Diatoms), chlorophyll concentration, F_v/F_m, σ_{PSII}, F_m:PsbA, Chl:PsbA, and dissolved iron concentrations (DFe). The strength of the linear association between each pair of variables is indicated by the colour of the square, with the negative and positive correlations denoted by '-' and '+' within all squares where significant ($p<0.01$).

Figure 4 Spatial distribution of $\Delta(F_v/F_m)$ calculated from Fe addition incubation experiments **(a)**. $\Delta(F_v/F_m)$ calculated from long-term Fe-addition incubation experiments in the Ross Sea, both from (1) near the Ross Ice Shelf, (2) over the Ross Bank and (3) within an anti-cyclonic eddy **(b)**. The change in chlorophyll normalized maximum fluorescence, (ΔF_m Chl^{-1}) from the three long-term Fe addition incubation experiments **(c)**. The change in chlorophyll normalized variable fluorescence $\Delta(F_v$ $Chl^{-1})$ from the three long-term Fe addition incubation

734 experiments **(d)**. Shown are averages with ± standard errors (*n* = 4 or 5). * represent
735 statistically significant differences (*t-test*, $p<0.05$).
736
737 **Figure 5** Relationship of DIN (μM) and photosynthetic efficiency (F_v/F_m) throughout the
738 Ross Sea as a function of a) chlorophyll concentrations (μg L^{-1}), b) phytoplankton
739 composition (%), and c) the relative degree of Fe stress $\Delta(F_v/F_m)$ **(c)**. Grey dots represent
740 stations where DIN and F_v/F_m were measured but no corresponding additional variables were
741 measured.
742
743 **Figure 6** Relationship of DIN (μM) and photosynthetic efficiency (F_v/F_m) throughout the
744 Ross Sea as a function of a) functional cross-section of photosystem II (σ_{PSII}) (nm^{-2}), b) the
745 ratio of chlorophyll to PsbA (a core subunit of PSII) (mmol mol^{-1}), c) the ratio of the
746 maximum fluorescence yield to chlorophyll (F_m:Chl), and d) the ratio of the maximum
747 fluorescence yield to PsbA (F_m:PsbA). Grey dots represent stations where DIN and F_v/F_m
748 were measured but no corresponding additional variables were measured.

749

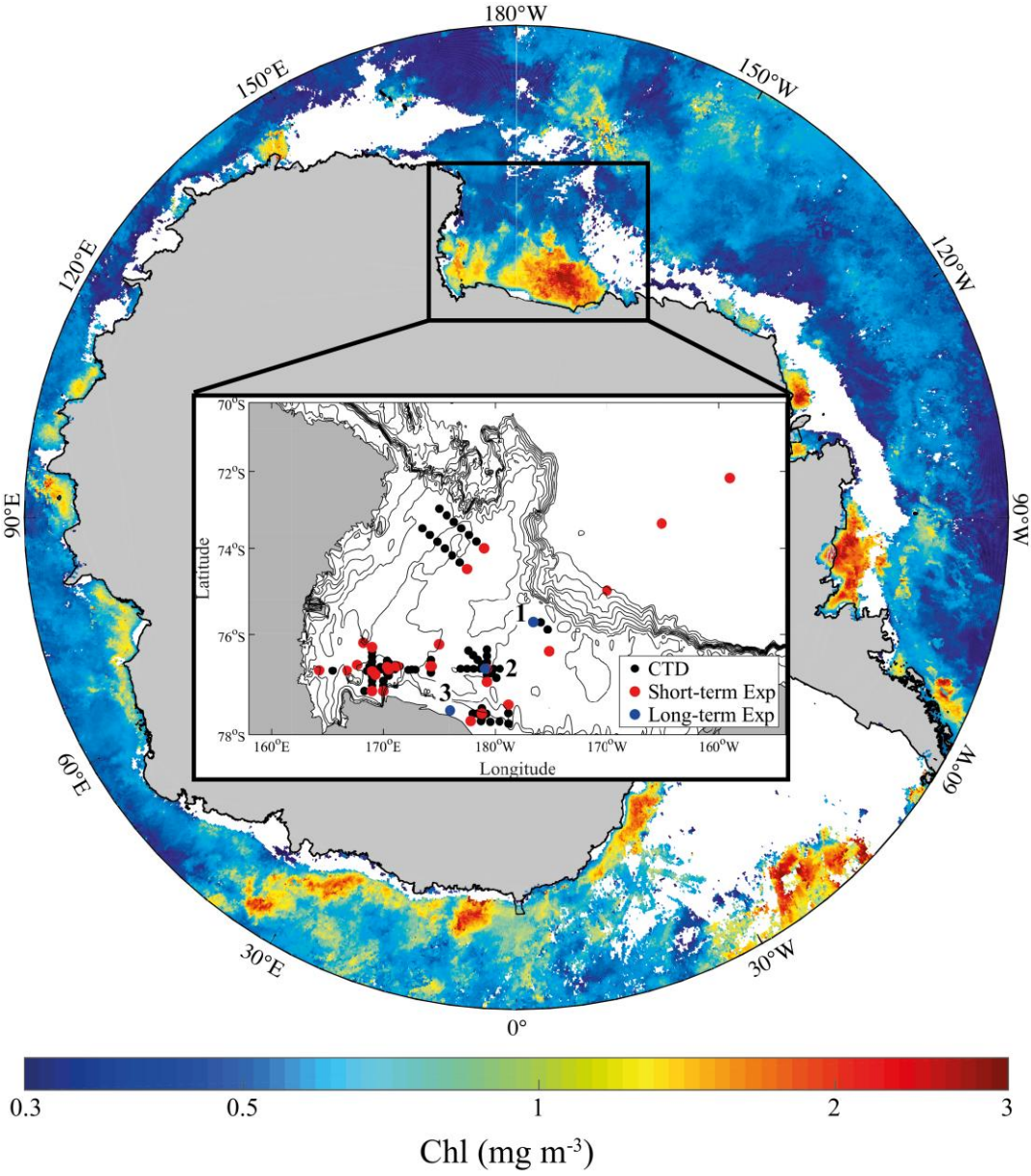
750 Table 1

751

Experiment	**1**	**2**	**3**
Lat (°S)	75.72	76.72	77.55
Long (°W)	183.40	179.08	175.97
$F_v/F_{m\ Initial}$	0.26 ±0.01	0.29 ±0.00	0.21 ±0.00
$\Delta (F_v/F_m)$, 24 h	0.04 ±0.01	0.00 ±0.00	0.01 ±0.00
$\mu^{Chl}_{Control}$ (d^{-1}), 0 -168 h	0.11 ±0.02	0.25 ±0.01	0.13 ±0.01
μ^{Chl}_{Fe} (d^{-1}), 0 -168 h	0.17* ±0.02	0.29* ±0.00	0.19* ±0.01
$\Delta NO_3^-{}_{Control}$ (μ M d^{-1}), 0 – 168 h	1.61 ±0.33	1.50 ±0.04	2.43 ±0.08
$\Delta NO_3^-{}_{Fe}$ (μ M d^{-1}), 0 – 168 h	2.53* ±0.13	1.57 ±0.05	2.93* ±0.07

752

753 Figure 1



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756 Figure 2

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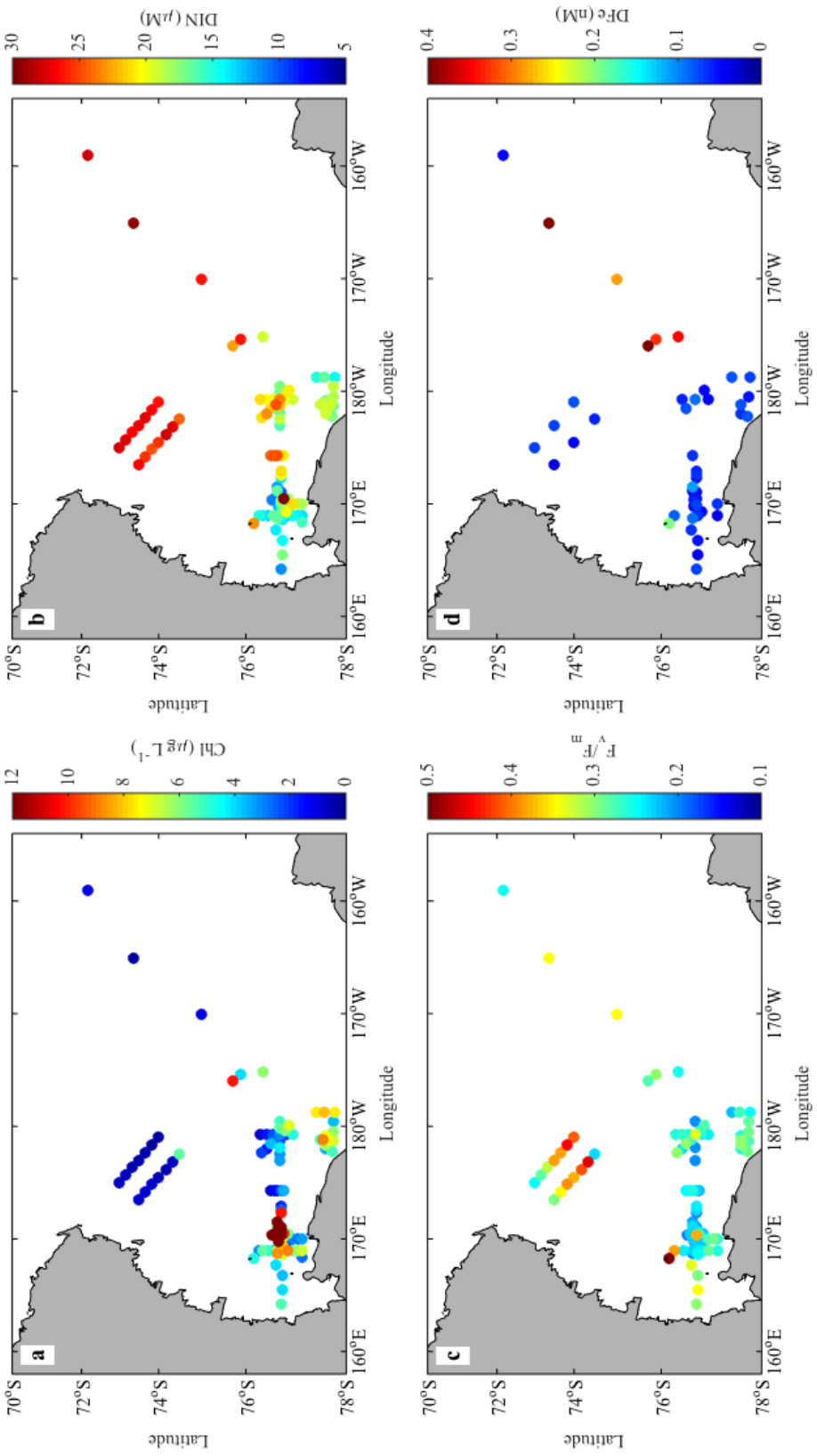

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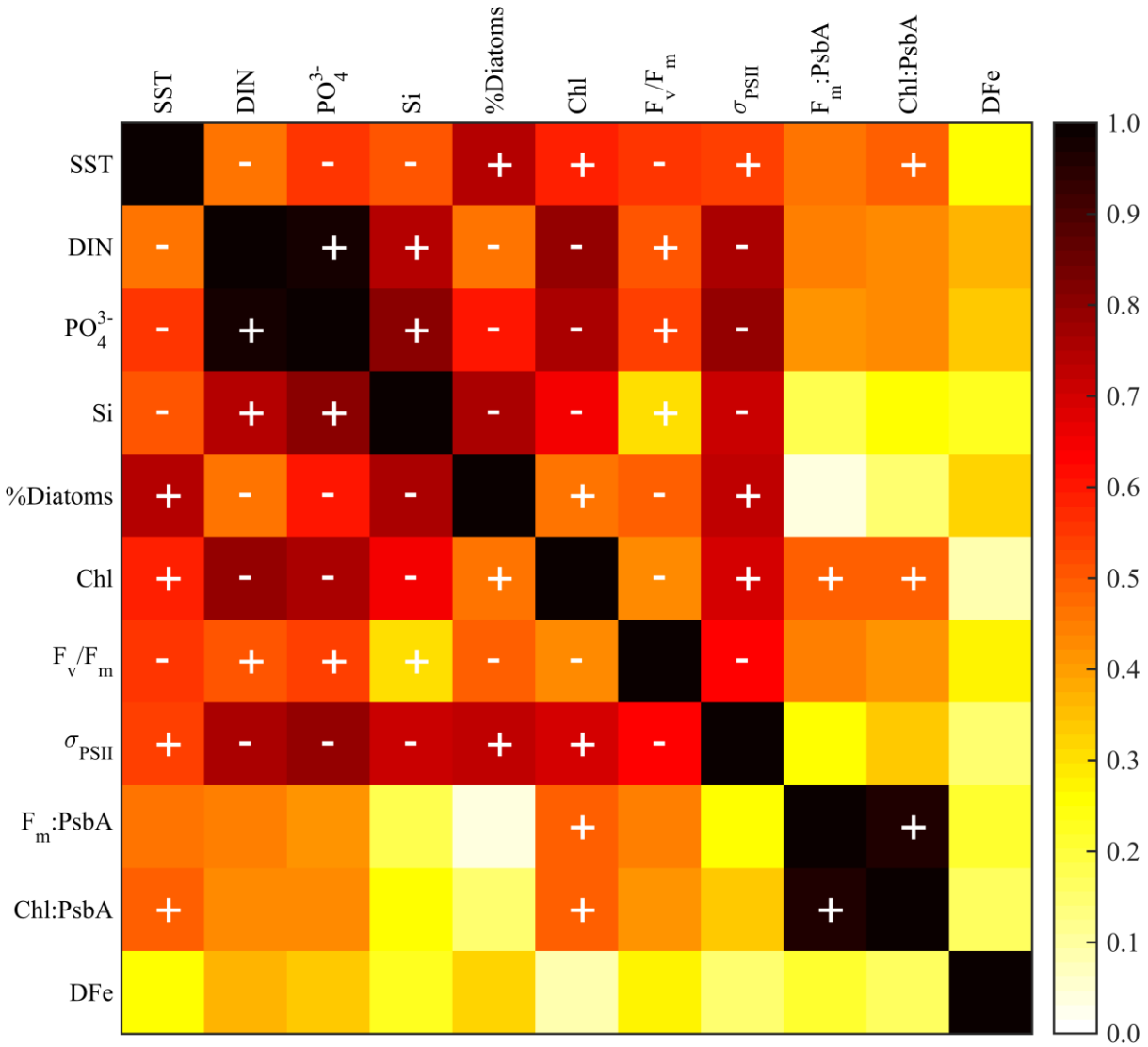

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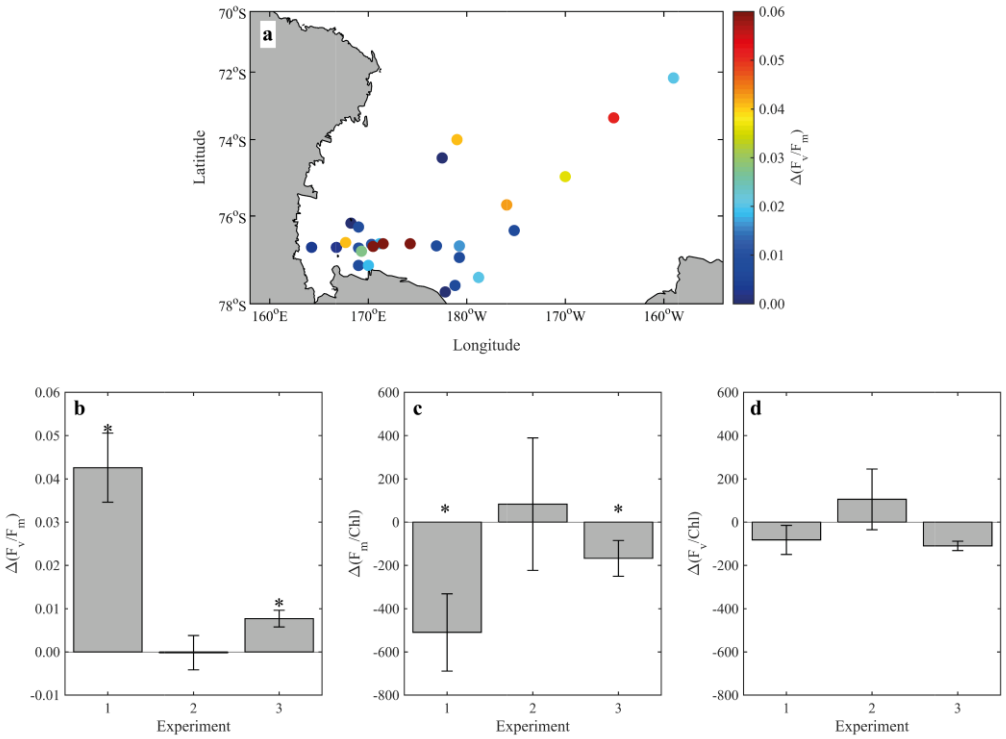

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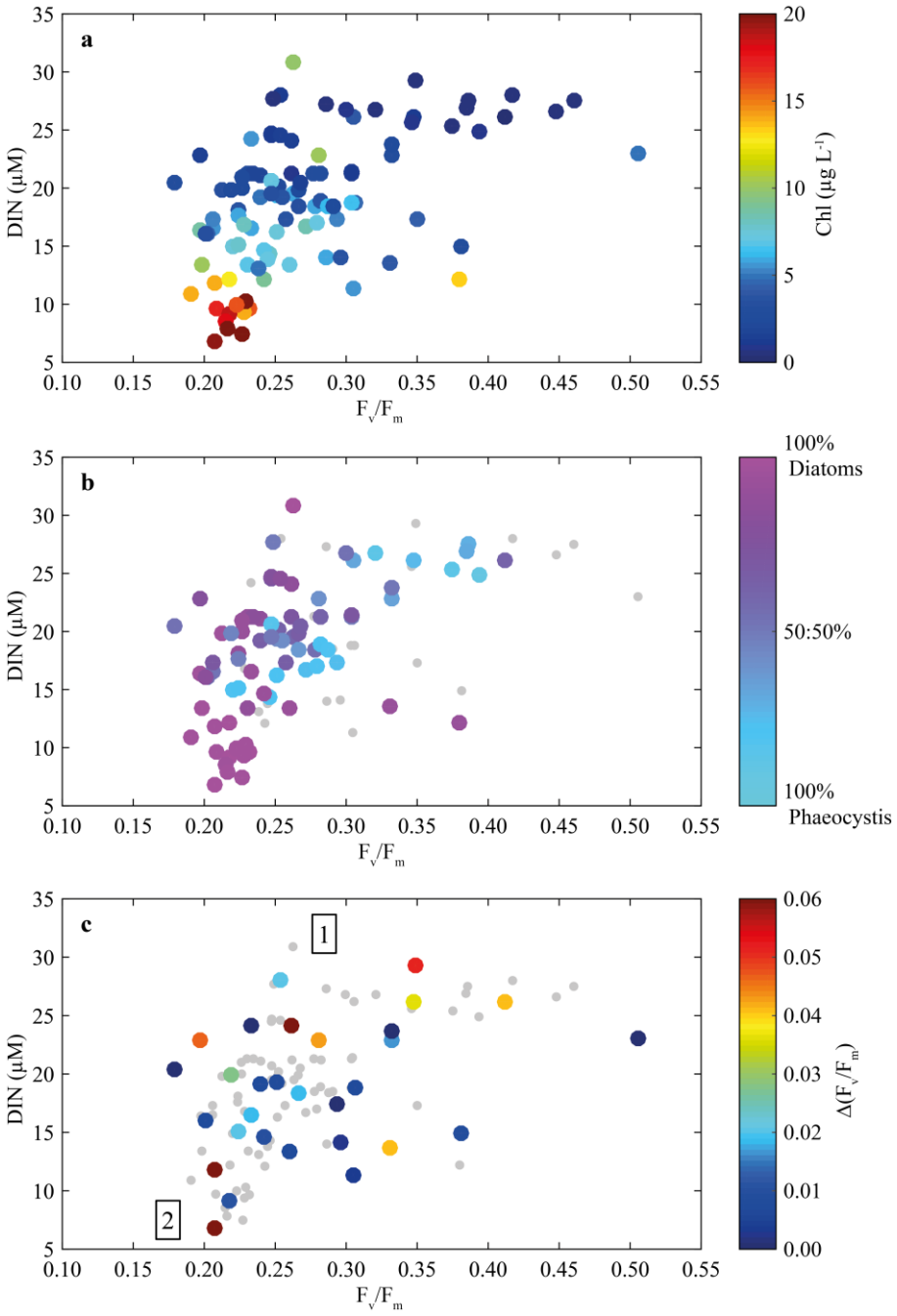

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768 Figure 6

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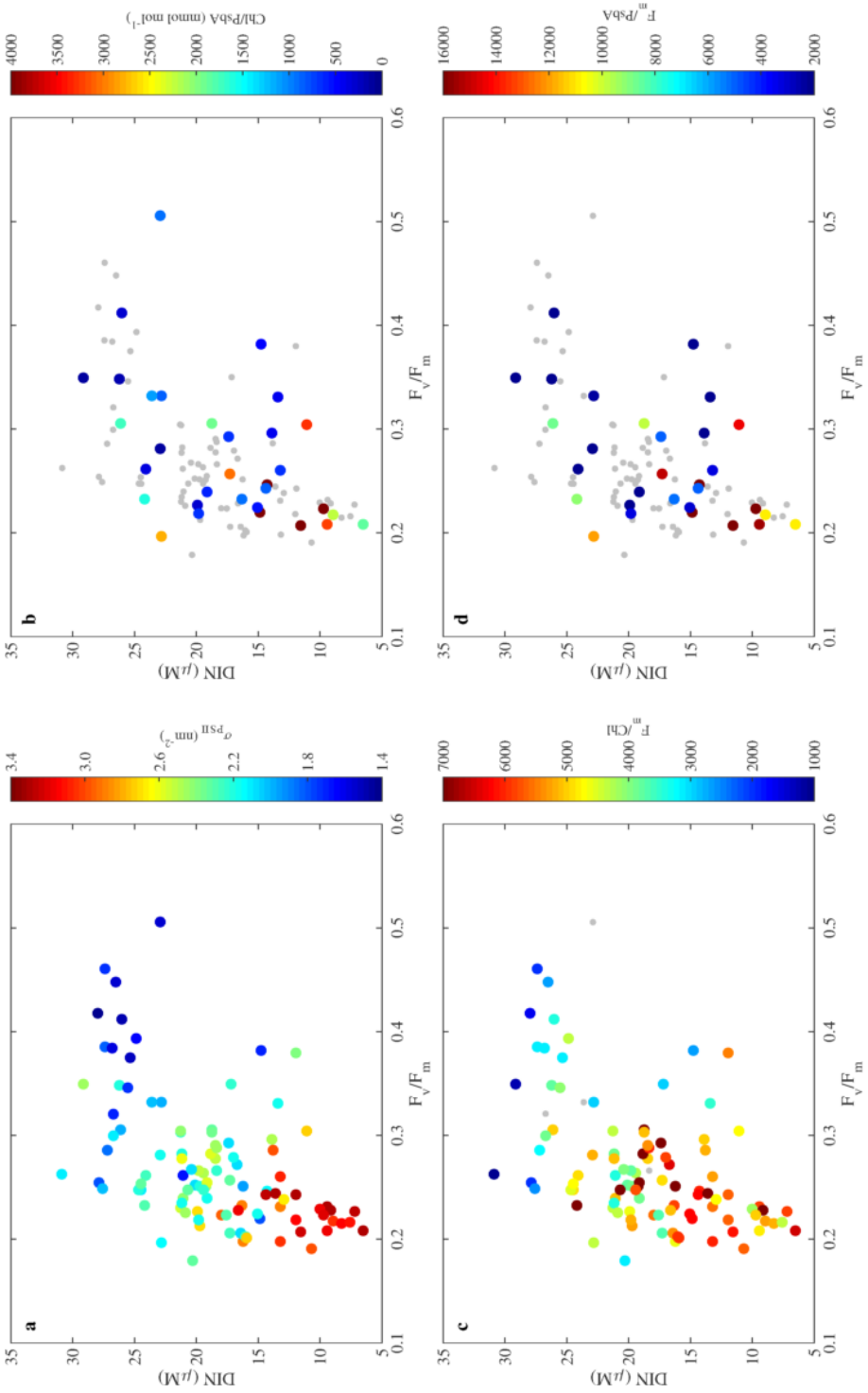

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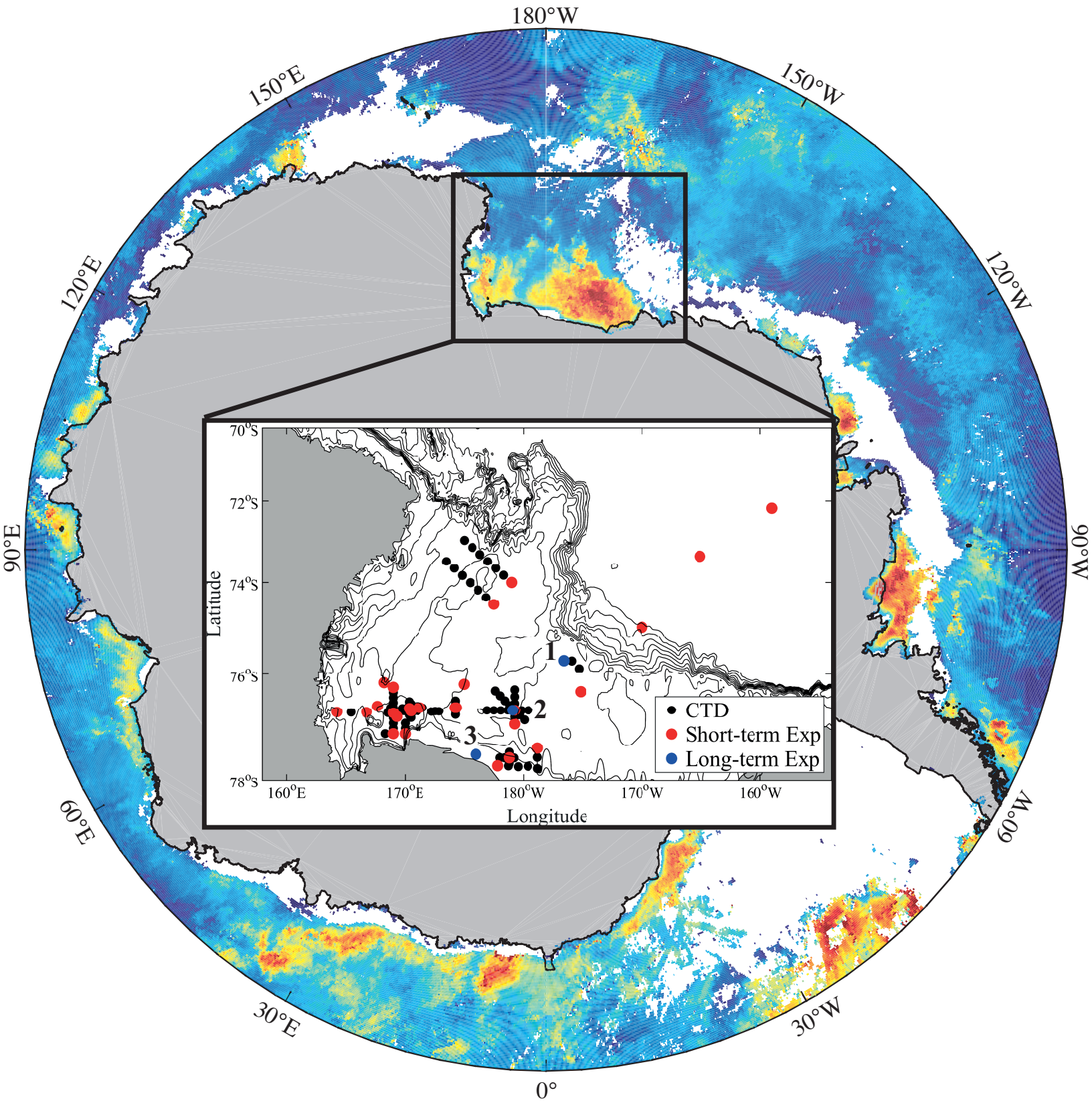

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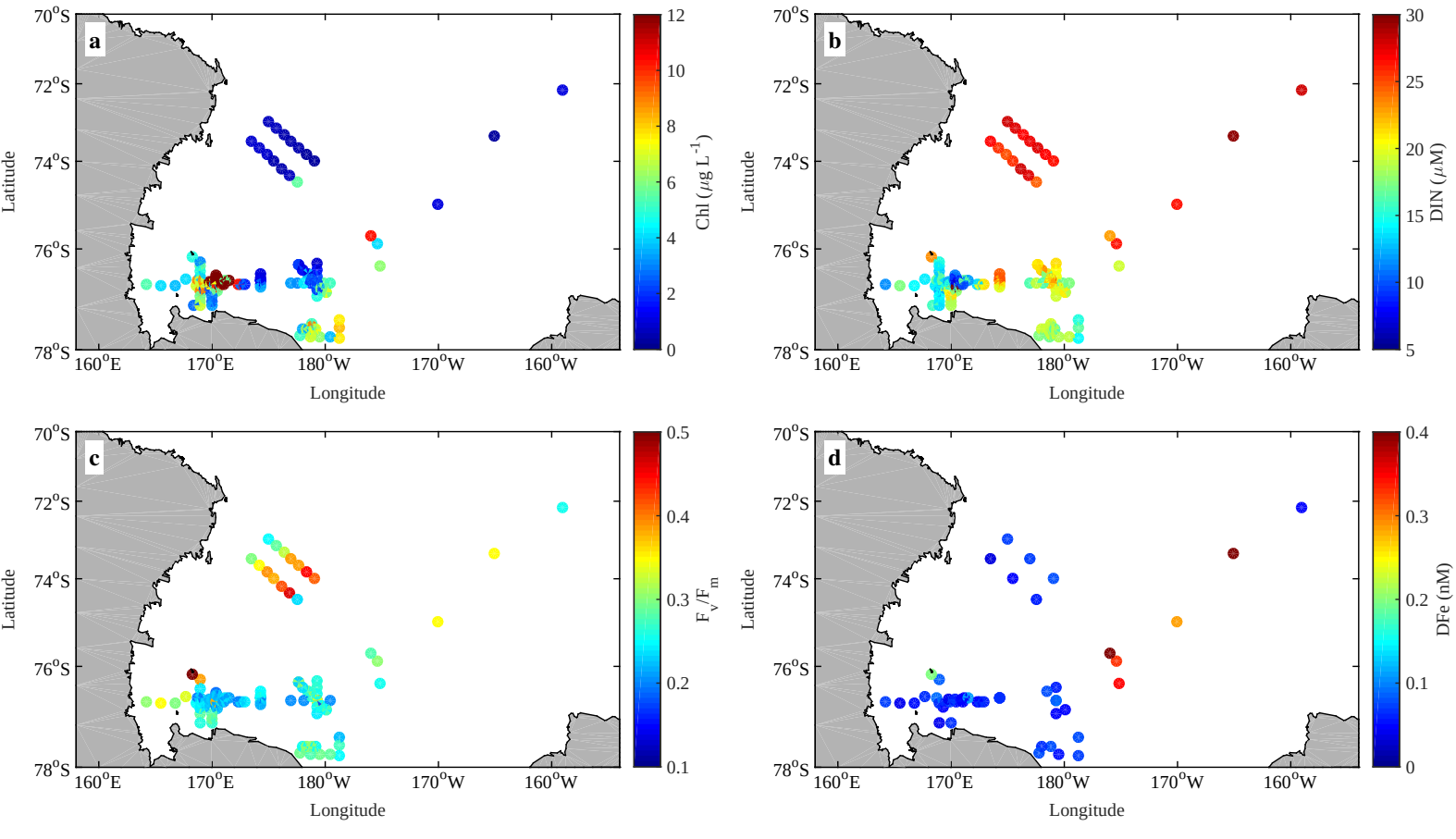

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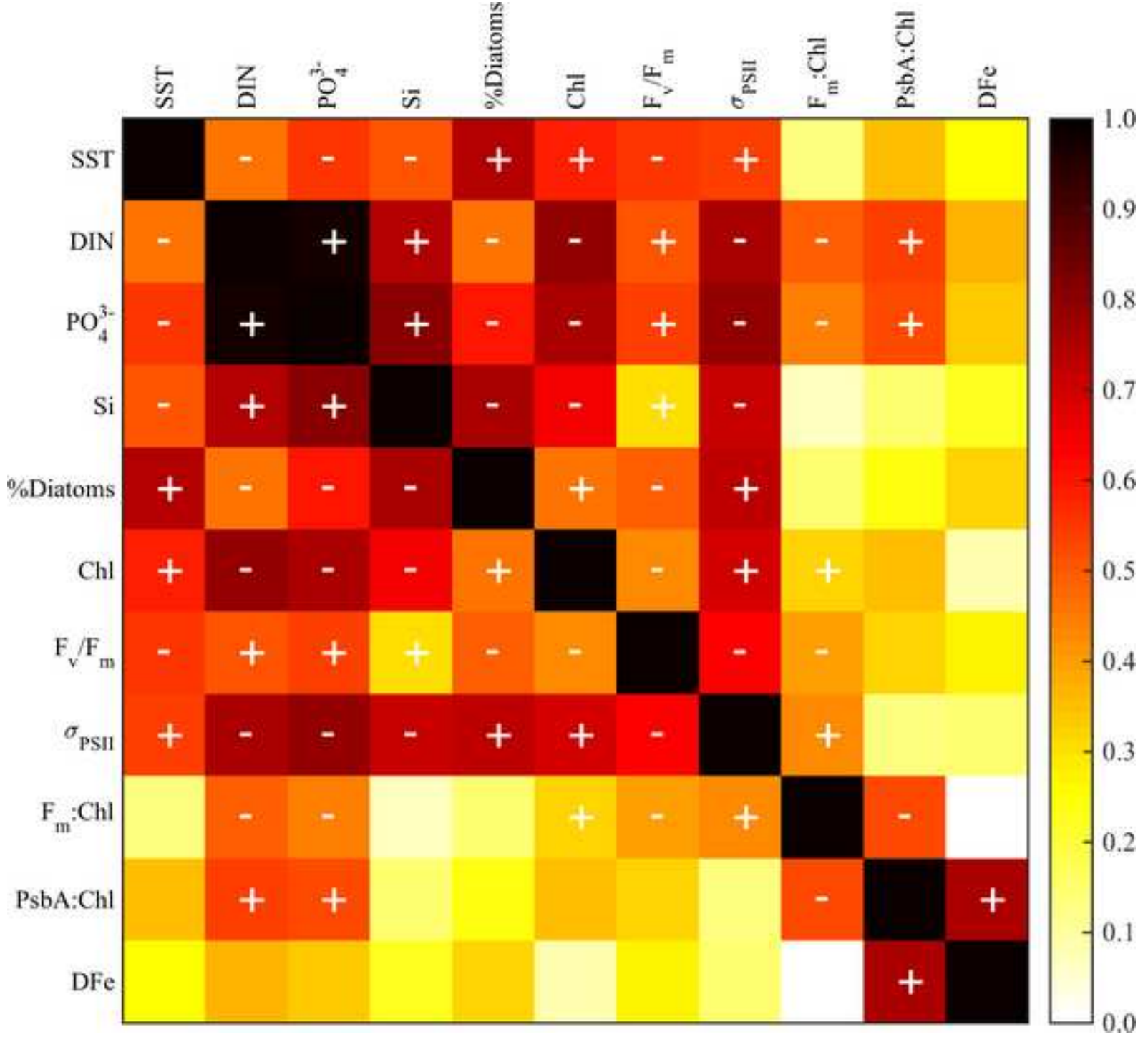

Figure 4

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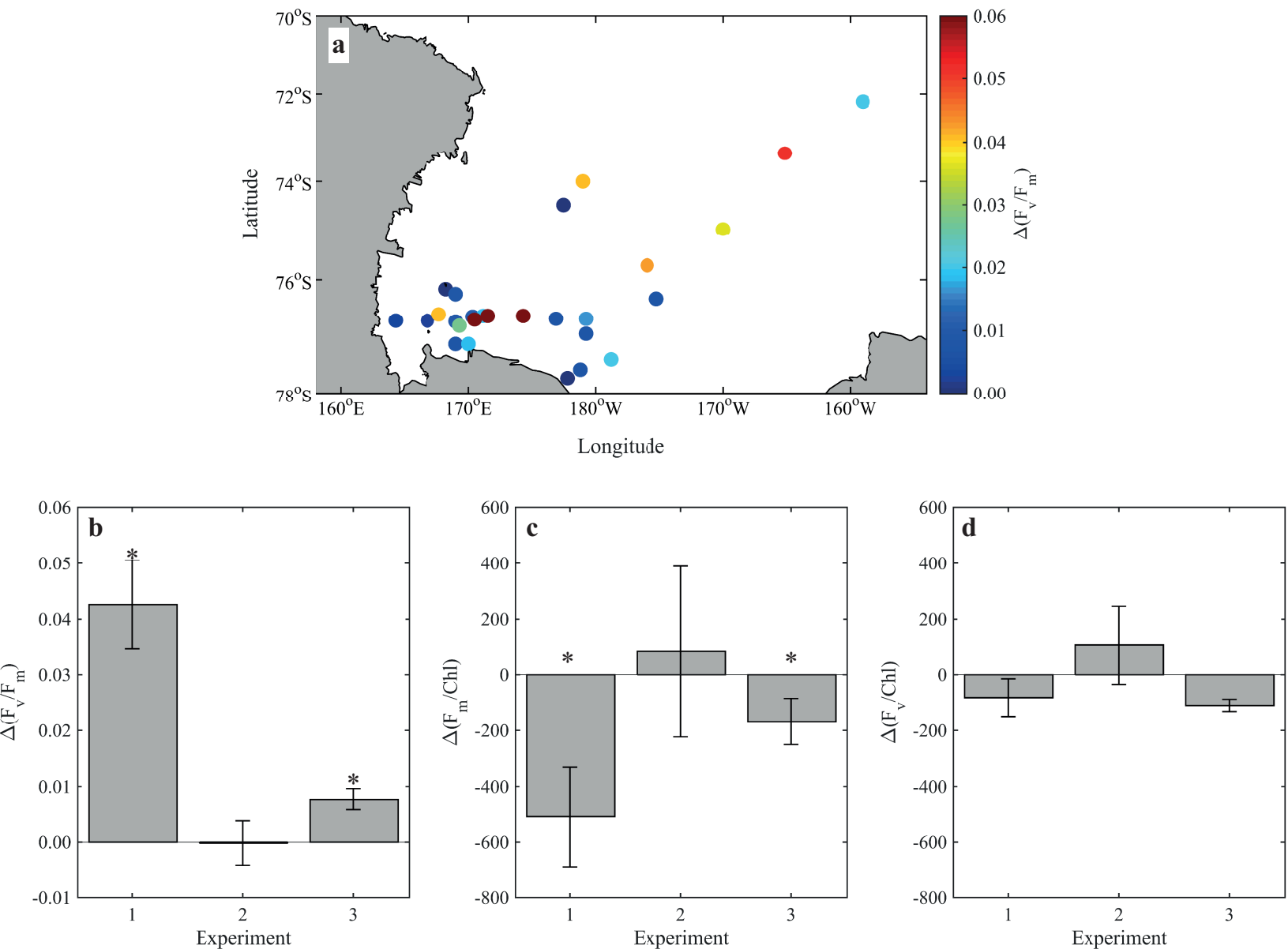

Figure 5

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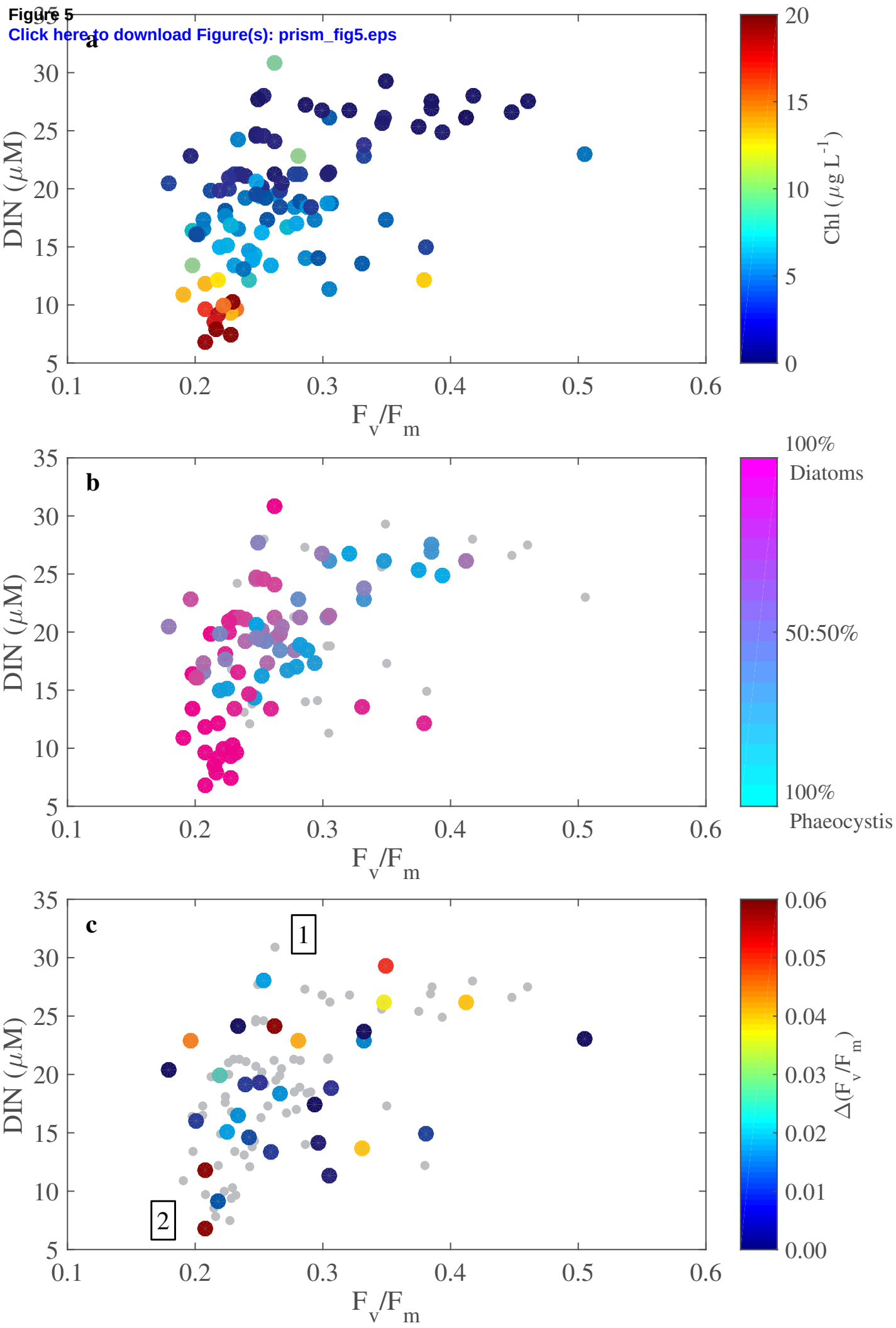

Figure 6

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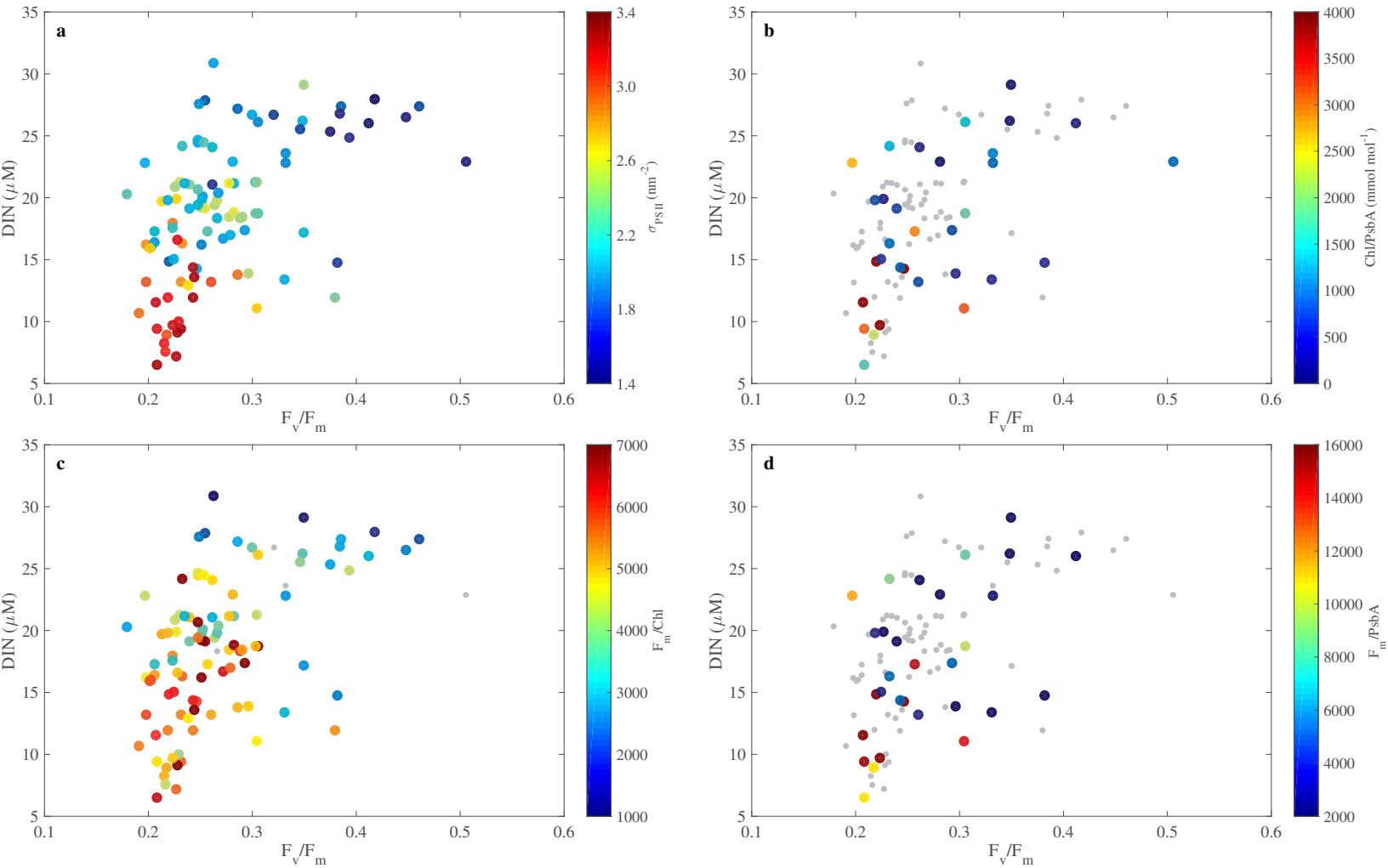

Figure S1

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