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# Stoichiometric N:P Ratios, Temperature, and Iron Impact Carbon and Nitrogen Uptake by Ross Sea Microbial Communities

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**Key Points:**
- Responses of Ross Sea microorganisms to changing dissolved N:P ratios, temperature, and Fe vary seasonally and among taxa
- Increasing the N:P ratio of the seawater increased nitrate uptake rates, but only over short time scales and during the late-season bloom
- Temperature elevation and Fe addition are more significant drivers of change than N:P supply ratios when *Phaeocystis* is dominant

**Supporting Information:**
- Supporting Information S1

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**Abstract** Phytoplankton growth in the seasonally productive Southern Ocean is typically limited by iron (Fe). In the next century, however, Fe inputs and temperature are predicted to increase. Dissolved concentrations of nitrogen (N) and phosphorus (P) may also change due to physical and biological drivers, altering the stoichiometric N:P ratio of the water column. Two separate experiments were conducted to study how these predicted changes will impact nutrient utilization by two natural microbial communities from the Ross Sea, Antarctica. The first investigation focused on a Terra Nova Bay community amended with Fe and grown under a wide range of seven different N:P ratios. Building on the results of the first study, a McMurdo Sound community was incubated under a factorial design at high, medium (ambient), and low N:P ratios, with and without Fe addition, and at ambient versus elevated temperature. In both experiments absolute uptake rates of bicarbonate, nitrate, and amino acids by two size fractions (0.7–5.0 μm and $>$ 5.0 μm) of microorganisms were measured using stable isotopes. For Terra Nova Bay microorganisms, significant differences in nutrient uptake rates occurred when the N:P ratio was elevated, and nitrate uptake rates significantly increased early in the experiment but were not different at the end. For McMurdo Sound microorganisms, changing the N:P supply ratio did not have a clear effect, while temperature elevation and/or Fe addition significantly increased nutrient uptake. Results indicate that changing the dissolved N:P ratio can potentially alter nutrient uptake rates; however, the impact of temperature and Fe are greater.

**Plain Language Summary** The Southern Ocean is one of the most biologically important ecosystems on our planet. Microscopic plants, called phytoplankton, form the base of the food web in the Southern Ocean and play a direct role in regulating how much and how fast elements like nitrogen and carbon are cycled throughout the world ocean. The goal of this research was to determine how predicted changes in the environment will impact how fast phytoplankton use these elements. The conditions that we tested included elevated temperature, addition of iron, and the proportion of nitrogen to phosphorus in the seawater. These parameters were selected because temperatures are increasing in the Southern Ocean, and the relative availability of nutrients can alter what species of phytoplankton are present and how fast they grow. Phytoplankton were collected from two locations in the Ross Sea, Antarctica, and grown for a few weeks under experimental conditions. Our results demonstrate that all three parameters, warmer temperatures, the addition of iron, and changing nitrogen to phosphorus ratios will increase how fast phytoplankton use nitrogen and carbon, but the impact of elevated temperature and the addition of iron had a much larger impact than the nitrogen to phosphorus ratio.

## 1. Introduction

Through measurements of the elemental composition of plankton and the concentration of nitrate ($NO_3^-$) and phosphate ($PO_4^{3-}$) in deep seawater samples, Redfield (1934) established that the nitrogen (N) to phosphorus (P) molar ratio in both marine plankton and the deep ocean nutrient pool was ~16:1. The Redfield ratio was later extended to include carbon (C) at a ratio of 106:16:1 (Fleming, 1940). The Redfield ratio essentially defines the *average* metabolic needs of plankton (Deutsch & Weber, 2012), and

it has been widely used as a fundamental principle to shape our understanding of marine ecology (Arrigo, 2005; Gruber & Deutsch, 2014). There are a number of processes that influence stoichiometric ratios in the marine environment, and research from the past few decades has concluded that ratios in the marine environment can range considerably outside of the Redfield ratio (Heckey et al., 1993; Geider & La Roche, 2002; Planavsky, 2014).

A few general trends describe the variability in stoichiometric ratios that are observed in the marine environment, both with dissolved nutrient ratios and elemental ratios of marine phytoplankton. First, C:N:P stoichiometry at the ecosystem level varies across time and space (DeVries & Deutsch, 2014; Martiny et al., 2013; Teng et al., 2014). Particulate C:P, N:P, and C:N ratios in high-latitude polar environments are typically below Redfield, in oligotrophic gyres these ratios are typically above Redfield, and in upwelling regions they are usually close to Redfield proportions (Martiny et al., 2013). We also know that evolutionary history influences the particulate C:N:P of eukaryotic phytoplankton with the green superfamily possessing significantly higher cellular ratios than the red superfamily (Quigg et al., 2003, 2011). Additionally, subtle stoichiometric differences are found among phytoplankton taxa. For example, diatoms typically have lower N:P ratios than other taxonomic groups (Deutsch & Weber, 2012; Planavsky, 2014), while haptophytes, such as *Phaeocystis antarctica*, have higher C:P and N:P ratios (Arrigo et al., 1999, 2000).

Although these general patterns exist, the cellular make up of marine phytoplankton is not homeostatic, and elemental ratios within the cell vary based on growth phase (Arrigo, 2005) and growth conditions (Goldman et al., 1979). Differences in elemental ratios occur during the cell cycle because certain cellular components become synthesized in higher proportions when cells are in exponential growth phase versus when they are approaching stationary phase and are growing more slowly due to limited resources. Ribosomal RNA, which is used for growth and is more abundant during exponential phase (Arrigo, 2005), has relatively low N:P ratios (Elser et al., 1996). In contrast, protein and chlorophyll, which are used to acquire resources and become more abundant during stationary phase (Arrigo, 2005), have high N:P ratios (Elser et al., 1996; Geider & La Roche, 2002). Factors that influence the physiology of phytoplankton cells may also influence elemental ratios. Temperature, for example, strongly affects cellular metabolic processes (Finkel et al., 2010) and can influence the N:P ratio at which phytoplankton growth is at its optimum (Tilman et al., 1986). Temperature also causes phytoplankton community shifts (Feng et al., 2009; Tatters et al., 2013), which can subsequently modify ecosystem C:N:P stoichiometry (Finkel et al., 2010).

Allochthonous delivery of nutrients has the potential to change the ratios of a system's dissolved nutrients, and altering the N:P supply ratio of an environment can favor certain species of phytoplankton (Lagus et al., 2004). Shifts in phytoplankton community composition due to stoichiometric changes in the nutrient pool have become increasingly common in coastal locations, where nutrient loading is a concern and where selective nutrient management is practiced (Cloern, 2001). The N:P supply ratio of more remote locations can also change. For example, the atmospheric deposition of anthropogenically-produced nitrous oxides can increase the availability of N (Pahlow & Riebesell, 2000), while P can be delivered via volcanic ash, aerosols, and mineral dust (Benitez-Nelson, 2000). Stoichiometric ratios can also be influenced through autochthonous mechanisms. Microbial pathways such as N fixation or denitrification, respectively, can increase or decrease dissolved N:P ratios by adding or removing N from the environment (Tyrell, 1999). Additionally, dissolved P can be removed through abiotic processes, increasing N:P ratios. Dissolved P can bind with iron (Fe) or humic-rich terrestrial organic matter that is delivered to coastal waters, and it can be removed through sediment burial (Paing et al., 1999; Paytan & McLaughlin, 2007).

Addition of micronutrients to the environment can also influence the elemental ratios of microorganisms. Studies using phytoplankton cultures find that conditions where Fe is replete sometimes yield higher particulate C:P and C:N ratios (Berman-Frank et al., 2001; De La Rocha et al., 2000; Dong et al., 2000), while others find the opposite trend (Doucette & Harrison, 1991; Maldonado & Price, 1996; Xu et al., 2014). The impact of Fe is particularly important to consider in regions like the Southern Ocean, where primary production is limited by extremely low Fe concentrations (De Baar et al., 1990), even in coastal regions (Bertrand et al., 2015). Sea surface temperatures in the Southern Ocean are predicted to increase by ~4 °C over the next century (Intergovernmental Panel on Climate Change, 2014), causing glaciers and icebergs to melt. Both of these are major sources of Fe to the Southern Ocean and are predicted to result in higher concentrations of Fe in the future (Hutchins & Boyd, 2016; Raiswell et al., 2008; Sedwick & DiTullio, 1997). Because the majority

of dissolved Fe is complexed with organic ligands, its accessibility to different phytoplankton groups will further depend on the chemical structure of the ligand (Gledhill & Buck, 2012; Hutchins et al., 1999).

Small changes to the nutrient ratios of an environment have the potential to significantly affect primary production (Karl et al., 1995) and the quality of food at the base of the food web, which have the potential to impact organisms at higher trophic levels (Sterner & Elser, 2002). For example copepod development appears to be stunted when its diet consists of phytoplankton that have been cultured under limiting N and P conditions (Klein Breteler et al., 2005). Because elemental stoichiometry of consumers varies among taxa, the dominant consumers present in an ecosystem can also influence dissolved nutrient ratios by preferentially holding onto some elements while excreting or metabolizing others (Anderson, 1994; Sterner & Elser, 2002).

Although our understanding of the mechanisms that govern elemental ratios in the marine environment has increased, we still lack a comprehensive view of how nutrient stoichiometry will be affected by global change and how subsequent shifts to microbial communities and N:P ratios will influence the utilization of nutrients by phytoplankton. Nutrient uptake and assimilation mediate the intrinsic link between dissolved nutrient ratios in the environment and cellular particulate ratios. Despite the critical role that nutrient uptake plays in the transfer of nutrients from the dissolved pool to the particulate pool, uptake rates are rarely measured in stoichiometric studies. Furthermore, the majority of studies investigating processes in the ocean that regulate stoichiometry have been focused on environments that are deplete in macronutrients, rather than in regions that have high concentrations of macronutrients such as the Southern Ocean. While general trends and patterns associated with the stoichiometry of the Southern Ocean have been identified, there have been very few controlled experiments specifically designed to assess how different dissolved N:P ratios impact Southern Ocean phytoplankton and the rates at which they take up nutrients. In this study, two experiments were conducted within the Ross Sea to determine how changing the dissolved N:P ratio of the seawater impacts uptake of inorganic and organic C and N substrates, particulate ratios, and phytoplankton community composition. The first experiment assessed the phytoplankton response to a wide range of dissolved N:P ratios when Fe limitation is removed. The second experiment, which was completed in a subsequent austral summer, built upon the first by employing a factorial design to determine the impact of select N:P ratios in conjunction with elevated temperature and Fe addition. Community composition was only assessed during the second experiment because community composition samples from the first experiment degraded prior to analysis.

## 2. Materials and Methods

### 2.1. Field Sampling

Field sampling occurred during the austral summer in 2013 and 2014 at different locations in the Ross Sea. Water for the first experiment was collected on 22 January 2013 from Silver Fish Bay within Terra Nova Bay (74°38′667″S, 164°49′896″E). For the second experiment, water was collected on 29 December 2014 from McMurdo Sound (77°37′4.62″S, 165°24′20.280″E); experiments will be referred to as TNB (Terra Nova Bay experiment) and MMS (McMurdo Sound experiment) henceforth. Sampling occurred at the ice edge, and water was collected from ~3- to 5 m depth using a trace metal clean diaphragm pump and Teflon tubing as previously described (Bertrand et al., 2015). Once the whole seawater was pumped to the surface, it was dispensed into acid washed (10% HCl) cubitainers, covered with dark plastic bags to exclude light, and directly transported to the laboratory at McMurdo Station by helicopter. We note that weather and extenuating logistical circumstances (e.g., the government shutdown) made it impossible for us to sample at the same time during the bloom season and at the same sites.

### 2.2. Bioassays

#### 2.2.1. TNB Experiment

Seawater was evenly distributed into six cubitainers (9 L; 10% HCl washed; 1 cubitainer was used to prepare each experimental condition), and either $NO_3^-$ or $PO_4^{3-}$ was added to manipulate the dissolved N:P ratio. During the austral summer, Fe limitation in the Ross Sea is common, with concentrations typically at approximately 0.1 nmol/L (Sedwick et al., 2011). To relieve potential Fe stress and ensure that we would see a response, all treatments, with the exception of the Control, also received an addition (+1 nmol/L) of iron (III) chloride ($FeCl_3$). A treatment that only received $FeCl_3$, but did not receive $NO_3^-$ or $PO_4^{3-}$, was also set up. The N:P stoichiometric ratios (determined as $NO_3^-$:$PO_4^{3-}$) that were tested included ~2, 11, 13, 33, and 49, all with added

**Table 1**
*Experimental Design of the TNB Experiment and the MMS Experiment*

| Treatment name | $+NO_3^-$ | $+PO4^{3-}$ | +Fe | Temperature (°C) | $NO_3^-$ (μmol N $L^{-1}$) | $PO_4^{3-}$ (μmol P $L^{-1}$) | $NO_3^-$:$PO_4^{3-}$ |
|---|---|---|---|---|---|---|---|
| | | | | TNB experiment | | | |
| 2* | — | Yes | Yes | 0 | 6.91 ± 0.0 | 3.29 ± 0.0 | 2.1 ± 0.0 |
| 11 (Control) | — | — | — | 0 | 6.71 ± 0.4 | 0.59 ± 0.0 | 11.3 ± 0.7 |
| 11* | — | — | Yes | 0 | 6.71 ± 0.4 | 0.59 ± 0.0 | 11.3 ± 0.7 |
| 13* | Yes | — | Yes | 0 | 7.68 ± 0.0 | 0.61 ± 0.0 | 12.6 ± 0.0 |
| 33* | Yes | — | Yes | 0 | 16.9 ± 0.0 | 0.51 ± 0.0 | 33.3 ± 0.0 |
| 49* | Yes | — | Yes | 0 | 25.0 ± 0.0 | 0.51 ± 0.0 | 49.0 ± 0.1 |
| | | | | MMS experiment | | | |
| Low | — | Yes | — | 0 | 19.4 ± 0.7 | 2.66 ± 0.1 | 7.3 ± 0.2 |
| Ambient (Control) | — | — | — | 0 | 18.7 ± 0.8 | 1.47 ± 0.1 | 12.8 ± 0.7 |
| High | Yes | — | — | 0 | 35.9 ± 8.5[a] | 1.28 ± 0.1[a] | 27.9 ± 5.0[a] |
| Low* | — | Yes | Yes | 0 | 19.7 ± 0.7 | 2.63 ± 0.2 | 7.5 ± 0.3 |
| Ambient* | — | — | Yes | 0 | 19.6 ± 0.6 | 1.41 ± 0.1 | 13.9 ± 0.4 |
| High* | Yes | — | Yes | 0 | 34.6 ± 0.5 | 1.57 ± 0.1 | 22.1 ± 1.0 |
| Low | — | Yes | — | 4 | 19.3 ± 0.6 | 2.63 ± 0.1 | 7.4 ± 0.4 |
| Ambient | — | — | — | 4 | 19.1 ± 0.8 | 1.54 ± 0.1 | 12.4 ± 0.0 |
| High | Yes | — | — | 4 | 33.5 ± 0.8 | 1.52 ± 0.0 | 22.1 ± 0.5 |
| Low* | — | Yes | Yes | 4 | 19.4 ± 0.7 | 2.63 ± 0.1 | 7.4 ± 0.3 |
| Ambient* | — | — | Yes | 4 | 19.3 ± 0.7 | 1.54 ± 0.1 | 12.5 ± 0.1 |
| High* | Yes | — | Yes | 4 | 34.4 ± 1.4 | 1.53 ± 0.1 | 22.6 ± 0.3 |

*Note*. The table shows the treatment name, if nitrate or phosphate was added ($+NO_3^-$, $+PO_4^{3-}$) to change the N:P ratio, if the treatment received an addition of iron (+Fe and the * in the treatment name also indicates added Fe), the incubation temperature, the intial $NO_3^-$ and $PO_4^{3-}$ concentrations in μmol N or P $L^{-1}$ ± 1 standard deviation, and the intitial $NO_3^-$:$PO_4^-$ ratio. TNB = Terra Nova Bay; MMS = McMurdo Sound.
[a]Nutrients were not collected on Day 0. Nutrient concentrations and the $NO_3^-$:$PO_4^{3-}$ ratio listed for the high treatment at 0 °C are from the Day 3 collection.

Fe, in addition to the control which had an N:P ratio of 11 (Table 1; note that treatments with added Fe are specified with an *). These ratios, although extreme at both ends, were selected so that we would be able to discriminate the physiological responses of the community over a wide range. After nutrients were added to the cubitainers, seawater was gently mixed and distributed into 2 L polyethylene terephthalate glycol-modified (PETG) bottles (filled to capacity 2.3 L). All treatments were prepared in triplicate with the exception of the Control, which was prepared in duplicate due to water limitations. Bottles were placed in an indoor incubator set at 0 °C that received ~45 μmol photons $m^{-2}$ $s^{-1}$ of constant light. This light level was selected because it is within light levels observed at ~3-m depth in open water and under the sea ice (McMinn et al., 2000). The community used in our experiment was collected from the ice edge; thus, the microorganisms would have likely experienced both open water and under ice light regimes within the recent past.

The experiment lasted 10 days. Chlorophyll *a*, $NO_3^-$ and $PO_4^{3-}$ concentrations were measured on Days 0, 3, 5, 7, 8, 9, and 10, and larger sampling events occurred on Days 0, 3, and 10. During larger sampling events, we also measured particulate C, particulate N, particulate P, bacterial abundance, nitrite ($NO_2^-$), ammonium ($NH_4^+$), urea, dissolved primary amines (DPA), total dissolved nitrogen (TDN), silicate (Si), and dissolved organic carbon (DOC). Additionally, during the large sampling events, uptake rates of $NO_3^-$ and dissolved inorganic carbon (DIC) were measured (described in detail below); on Day 0 and Day 10 uptake rates of amino acids were also measured. We note that limits on available water precluded measuring all parameters at all time points for both the TNB and MMS experiments.

A different experimental design was employed for the MMS experiment to build on the results of the TNB experiment. We learned from the TNB experiment that different dissolved N:P ratios have the potential to impact nutrient uptake (results presented below); however, we were not able to conclusively determine the impact of Fe addition at different N:P ratios. Furthermore, studies conducted on isolates from the first season showed that temperature elevation has a strong impact on the physiology of dominant phytoplankton taxa from the Ross Sea (Zhu et al., 2016). Thus, additional parameters were included in the MMS study described below.

#### 2.2.2. MMS Experiment

Seawater was distributed into 2 L PETG bottles (filled to capacity 2.3 L), and nutrient amendments were made using $NO_3^-$, $PO_4^{3-}$, and $FeCl_3$. The $NO_3^-$ and $PO_4^{3-}$ was chelated to remove metals as described in Sunda et al. (2005), and the $FeCl_3$ was prepared according to Bertrand et al. (2015). A factorial design was used to examine two temperature levels (0 °C and 4 °C), decreased N:P ratios (~7.4 and designated as Low), unaltered N:P ratios (~13 and designated as Ambient), increased N:P ratios (~22 and designated as High), and Fe addition (+2 nmol/L; treatments with added Fe are distinguished with an *; Table 1). Bottles were incubated for 7 days in two indoor incubators (one for each temperature) under constant light (~75–100 μmol photons $m^{-2}$ $s^{-1}$). A slightly higher irradiance level was used in this experiment compared to the TNB experiment, because this experiment occurred earlier in the austral summer when light levels are typically higher. Chlorophyll *a*, $NO_3^-$, and $PO_4^-$ concentrations were measured on Days 3 and 5. Larger sampling events occurred on Days 0 and 7, and on these days the same parameters listed above for the TNB experiment (large sampling events) were measured, and phytoplankton community composition was also analyzed via microscopy.

### 2.3. Uptake Experiments

Uptake rates of C and N substrates were measured using $^{13}C$ and $^{15}N$ stable isotope tracer techniques. Substrates included $^{13}C$-labeled sodium bicarbonate ($NaH^{13}CO_3^-$; 99%), $^{15}N$-labeled potassium nitrate ($K^{15}NO_3$; 98%), and a $^{13}C$- and $^{15}N$-labeled algal amino acid mixture comprised of 16 amino acids, specified as either AA-C or AA-N (97–99%; all substrates came from Cambridge Isotope Laboratories, Andover, MA). Uptake rates of bicarbonate were used as a measure of primary productivity, which is directly coupled to global N and P cycles. Primary production can both affect and be affected by changes in nutrient availability. Uptake of $NO_3^-$ was assessed because it is seasonally replete in the Southern Ocean and was the N substrate used to manipulate the N:P ratio in this experiment. Algal derived amino acids are a mix of labile organic C and N containing compounds that are released as primary production byproducts. *Phaeocystis*, a prominent species of phytoplankton in the Ross Sea, also produces mucus that contains amino sugars (Solomon et al., 2003), which can be converted to amino acids through bacterial degradation (Mulholland & Lomas, 2008). Due to the timing of these studies, amino acids were used to assess the uptake of labile bloom products.

Uptake experiments were done in acid washed 500-ml PETG bottles filled to capacity (~600 ml) with the exception of the TNB Day 10 amino acid uptake experiment and the MMS Day 0 uptake experiment, where 250 ml bottles and 1 L bottles were used, respectively. Uptake experiments on Day 0 were conducted using the seawater that had been brought back from each site (TNB and MMS), and uptake experiments at subsequent time points used water subsampled from each experimental bottle. Once $^{13}C$- and $^{15}N$-labeled substrates had been added at an estimated 10% enrichment of background concentrations, bottles were returned to their respective incubators and incubated for ~6 hr. Uptake rates of two microbial size fractions were determined by collecting the larger size fraction (>5.0 μm) on a Sterlitech silver membrane filter, collecting the filtrate, and then passing the filtrate through a GF/F filter to collect the smaller size fraction (0.7–5.0 μm). Retention of Ross Sea bacteria on GF/F filters was tested and found to be 88.8%. Samples were kept frozen (−40 °C) until analysis on a Europa 20/20 isotope ratio mass spectrometer. Absolute uptake rates for $^{13}C$-labeled $HCO_3^-$ and $^{15}N$-labeled $NO_3^-$ were calculated according to Hama et al. (1983) and Dugdale and Wilkerson (1986), respectively; $HCO_3^-$ uptake will be referred to as DIC uptake henceforth. The same approach was used to calculate C and N uptake rates for dual-labeled amino acids (Bronk et al., 2014). Uptake rates are reported as separate size fractions (0.7–5.0 μm; >5.0 μm); for chlorophyll *a* concentrations and particulate ratios, the two size fractions were added together and thus represent the >0.7 μm community.

### 2.4. Dissolved Nutrient Analyses

To measure dissolved nutrient concentrations, seawater was filtered through a Whatman GF/F filter (combusted at 450 °C for 2 hr) and the filtrate was collected. A Lachat QuickChem 8500 autoanalyzer was used to measure $NO_3^-$, $NO_2^-$, $PO_4^{3-}$, and Si concentrations in duplicate (detection limit (DL) 0.03 μmol N $L^{-1}$, DL 0.03 μmol P $L^{-1}$, DL 0.05 μmol Si $L^{-1}$; Parsons et al., 1984). A Shimadzu UV-1601 spectrophotometer was used to measure concentrations of $NH_4^+$ in triplicate and concentrations of urea in duplicate using the phenol-hypochlorite method (DL 0.05 μmol N $L^{-1}$; Koroleff, 1983) and the manual diacetyl monoxime

thiosemicarbazide method (DL 0.025 μmol N $L^{-1}$; Price & Harrison, 1987), respectively. A Shimadzu RF-1501 spectrofluorometer was used to measure concentrations of DPA in triplicate according to the OPA (*o*-phthaldialdehyde) method (DL 0.025 μmol N $L^{-1}$; Parsons et al., 1984). A Shimadzu 5000A TOC-V/TNM analyzer was used to measure TDN and dissolved organic carbon (DOC; DL 2 μmol N $L^{-1}$, 5 μmol C $L^{-1}$; Sharp et al., 2004); analytical accuracy was ensured by using deep-sea reference water samples (University of Miami consensus reference material program, Hansell, 2005). Coulometry (CM5230, UIC) was used to measure concentrations of DIC using the method described in King et al. (2011).

### 2.5. Particulate Parameters

Concentrations of particulate C and N were measured on a GEO 20/20 isotope ratio mass spectrometer at the same time that $^{13}C$ and $^{15}N$ enrichments were measured. Particulate P samples were baked for 2 hr at 550 °C, extracted overnight using hydrochloric acid (1 N), and concentrations of orthophosphate were analyzed colorimetrically (Aspila & Agemian, 1976). Bacterial abundance samples were preserved with paraformaldehyde (final concentration of 0.5%) and then flash frozen and stored at −80 °C. For enumeration, bacteria samples were stained using SYBR Green (Invitrogen) and counted on a BD Influx high-speed sorting flow cytometer. Chlorophyll *a* samples were collected on GF/F filters and polycarbonate filters (nominal pore size 5.0 μm), extracted overnight using 90% acetone, and measured using the nonacidified fluorometric method on a Turner Designs fluorometer (Welshmeyer, 1994). Samples for phytoplankton cell counts were preserved using glutaraldehyde and acidified Lugol's iodine solution, stored at 4 °C in the dark, settled in a counting chamber, and enumerated microscopically using an Accu-Scope 3032 (Utermöhl, 1931). Species were identified according to Scott and Marchant (2005) and Tomas (1997). For the MMS experiment, the most common phytoplankton taxa were identified, and the relative abundance of these taxa are expressed as the percent of all phytoplankton cells counted. Taxa include diatoms (*Pseudo-nitzschia* spp., *Fragilariopsis* spp., other pennate diatoms, centric diatoms), dinoflagellates, *Phaeocystis* (solitary cells and colonial cells), and other (all other taxa, mostly rare species). The number of cells comprising *Phaeocystis* colonies was determined; hence, the number of individual colonial cells were included in the relative abundance calculation. Individual cells associated with *Phaeocystis* colonies were determined by counting the number of cells in each colony if they could be assessed. If the number of cells per colony could not be determined visually, the colony was dissolved using EDTA, vortexed to extract the cells from the colony matrix, and then enumerated (Rousseau et al., 1990). The number of colonies in each sample was also noted.

### 2.6. Data Analysis and Statistics

All statistical analyses were completed using the open source RStudio program version 0.99.490 (RStudio Team, 2015). Data were checked for normality using the Shapiro-Wilk test, and if data were not normally distributed, they were log transformed prior to statistical analysis. Two-way and multiway analysis of variances were used to determine if there were significant differences between treatments, and post hoc Tukey's Tests were used to identify means that were significantly different from one another. If p values were $\leq 0.05$, means were considered significantly different from one another.

## 3. Results and Discussion

### 3.1. TNB Experiment

The TNB experiment was performed using a microbial assemblage collected late in the growing season. At the TNB site (Table 2), the TDN pool was comprised primarily of DON (~51%) followed by $NO_3^-$ (~34%). The concentration of $PO_4^{3-}$ was less than 1 μmol P $L^{-1}$, and the $NO_3^-$:$PO_4^{3-}$ ratio was ~11.3. The dissolved $NO_3^-$:$PO_4^{3-}$ ratios remained within ~2 units of the target ratio throughout the experiment (Tables 1 and 2). Bulk DON concentrations decreased by nearly half of the initial concentration in all treatments over the course of the experiment. Concentrations of urea and DPA (components of the bulk DON pool), however, slightly increased in all treatments. These results likely indicate that the microbial communities were readily using and recycling DON. Concentrations of Si remained constant during the experiment. Comparing the treatments, the lowest nutrient concentrations generally occurred when only Fe was added (11* treatment). In the 11* treatment, drawdown of both $NO_3^-$ and $PO_4^{3-}$ was triple that of the Control, and particulate N and P concentrations were significantly higher in the 11* treatment compared to the Control on Day 10 ($p < 0.04$; Table S1), which indicates that the original community was limited by Fe. We also note that $NO_3^-$ and $PO_4^{3-}$

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**Table 2**
*Nutrient Concentrations of the Initial Seawater From Each Site and of the Treatments on the Final Day of Each Experiment*

| | | DOC | $NH_4^+$ | $NO_2^-$ | $NO_3^-$ | DON | Urea | DPA | $PO_4^{3-}$ | Si | $NO_3^-$:$PO_4^{3-}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | μmol C $L^{-1}$ | μmol N $L^{-1}$ | μmol N $L^{-1}$ | μmol N $L^{-1}$ | μmol N $L^{-1}$ | μmol N $L^{-1}$ | μmol N $L^{-1}$ | μmol P $L^{-1}$ | μmol Si $L^{-1}$ | |
| TNB Site Water | | | | | | | | | | | |
| | | 62.9 ± 2.1 | 2.89 ± 0.0 | 0.13 ± 0.0 | 6.71 ± 0.4 | 10.1 ± 1.7 | 0.81 ± 0.0 | 0.25 ± 0.0 | 0.59 ± 0.0 | 32.4 ± 0.3 | 11.3 ± 0.7 |
| TNB Treatments (Day 10) | | | | | | | | | | | |
| | 2* | 61.6 ± 3.0 | 2.26 ± 0.2 | 0.12 ± 0.0 | 5.74 ± 0.1 | 4.42 ± 0.8 | 0.99 ± 0.0 | 0.27 ± 0.0 | 3.2 ± 0.0 | 32.5 ± 0.8 | 1.8 ± 0.1 |
| | 11 (Control) | 67.0 ± 0.0 | 2.80 ± 0.0 | 0.11 ± 0.0 | 6.35 ± 0.1 | 4.95 ± 0.2 | 0.93 ± 0.1 | 0.31 ± 0.0 | 0.57 ± 0.0 | 32.2 ± 0.1 | 11.2 ± 0.0 |
| | 11* | 57.3 ± 0.8 | 1.68 ± 0.4 | 0.12 ± 0.0 | 5.58 ± 0.3 | 4.28 ± 1.1 | 0.87 ± 0.1 | 0.29 ± 0.1 | 0.47 ± 0.1 | 31.0 ± 1.8 | 12.0 ± 0.7 |
| | 13* | 65.1 ± 3.2 | 2.12 ± 0.1 | 0.12 ± 0.0 | 6.45 ± 0.1 | 5.76 ± 0.2 | 0.93 ± 0.0 | 0.34 ± 0.0 | 0.52 ± 0.0 | 32.2 ± 0.3 | 12.4 ± 0.3 |
| | 33* | 64.9 ± 1.5 | 1.87 ± 0.2 | 0.13 ± 0.0 | 15.3 ± 0.1 | 5.68 ± 0.8 | 0.95 ± 0.1 | 0.38 ± 0.1 | 0.48 ± 0.0 | 31.5 ± 1.3 | 31.4 ± 0.4 |
| | 49* | 68.1 ± 1.7 | 2.43 ± 0.1 | 0.13 ± 0.0 | 24.8 ± 0.1 | 5.62 ± 0.4 | 0.97 ± 0.0 | 0.31 ± 0.0 | 0.50 ± 0.0 | 33.7 ± 0.1 | 49.4 ± 0.2 |
| MMS Site Water | | | | | | | | | | | |
| | | 60.9 ± 2.5 | 1.04 ± 0.4 | BD | 20.9 ± 0.1 | BD | 0.22 ± 0.0 | 0.06 ± 0.0 | 1.40 ± 0.0 | 69.6 ± 0.6 | 14.9 ± 0.1 |
| MMS Treatments (Day 7) | | | | | | | | | | | |
| 0 °C treatments | Low | 61.3 ± 1.7 | 0.60 ± 0.0 | BD | 14.9 ± 1.0 | 2.73 ± 1.8 | 0.11 ± 0.0 | 0.19 ± 0.0 | 2.17 ± 0.2 | 69.7 ± 0.5 | 6.9 ± 0.8 |
| | Ambient (Control) | 58.7 ± 0.7 | 0.69 ± 0.1 | BD | 14.3 ± 1.3 | 3.43 ± 1.6 | 0.13 ± 0.0 | 0.19 ± 0.0 | 1.16 ± 0.2 | 70.6 ± 0.9 | 12.6 ± 2.8 |
| | High | 63.9 ± 1.5 | 0.51 ± 0.0 | BD | 35.7 ± 9.2 | 2.25 ± 13 | 0.12 ± 0.0 | 0.18 ± 0.0 | 1.12 ± 0.1 | 69.1 ± 0.2 | 32.7 ± 12.3 |
| | Low* | 69.5 ± 0.2 | 0.59 ± 0.0 | BD | 13.2 ± 1.4 | 2.49 ± 1.9 | 0.17 ± 0.1 | 0.26 ± 0.0 | 2.15 ± 0.3 | 64.4 ± 6.5 | 6.2 ± 0.5 |
| | Ambient* | 67.1 ± 3.8 | 0.60 ± 0.0 | BD | 13.0 ± 0.4 | 2.75 ± 0.5 | 0.13 ± 0.0 | 0.24 ± 0.0 | 1.11 ± 0.0 | 68.4 ± 0.9 | 11.7 ± 0.3 |
| | High* | 72.3 ± 5.0 | 0.54 ± 0.0 | BD | 27.5 ± 1.1 | 0.83 ± 1.2 | 0.15 ± 0.0 | 0.26 ± 0.0 | 1.16 ± 0.0 | 69.8 ± 0.4 | 23.7 ± 0.1 |
| 4 °C treatments | Low | 79.9 ± 6.2 | 0.62 ± 0.1 | BD | 14.6 ± 0.8 | 0.63 ± 1.9 | 0.08 ± 0.0 | 0.20 ± 0.0 | 2.17 ± 0.3 | 70.8 ± 0.8 | 6.8 ± 1.5 |
| | Ambient | 76.8 ± 4.0 | 0.63 ± 0.0 | BD | 15.5 ± 0.5 | 1.34 ± 0.5 | 0.11 ± 0.0 | 0.17 ± 0.0 | 1.29 ± 0.0 | 69.9 ± 0.4 | 12.0 ± 0.3 |
| | High | 81.0 ± 5.1 | 0.55 ± 0.0 | BD | 29.0 ± 1.1 | BD | 0.05 ± 0.0 | 0.18 ± 0.0 | 1.28 ± 0.0 | 67.9 ± 2.2 | 22.6 ± 0.6 |
| | Low* | 37.8 ± 12.8 | 0.61 ± 0.1 | BD | 10.6 ± 0.7 | BD | 0.11 ± 0.0 | 0.41 ± 0.2 | 2.21 ± 0.2 | 71.6 ± 2.8 | 4.9 ± 0.8 |
| | Ambient* | 88.7 ± 3.5 | 0.60 ± 0.0 | BD | 11.7 ± 0.8 | 1.28 ± 1.4 | 0.09 ± 0.0 | 0.25 ± 0.0 | 0.93 ± 0.2 | 67.9 ± 1.1 | 12.8 ± 1.9 |
| | High* | 82.9 ± 5.0 | 0.56 ± 0.0 | BD | 24.2 ± 1.1 | 0.32 ± 2.7 | 0.06 ± 0.0 | 0.46 ± 0.3 | 1.03 ± 0.0 | 66.5 ± 2.7 | 23.5 ± 0.7 |

*Note*. Nutrient concentrations of the seawater collected from Terra Nova Bay (TNB site water) and McMurdo Sound (MMS site water), and nutrient concentrations measured on the final day of the two experiments for each treatment. Concentrations are in units of μmol C, N, P, or Si $L^{-1}$ ± 1 standard deviation ($n$ = 3). BD = below detection. Treatment names describe either the N:P ratio (TNB experiment) or whether the N:P ratio was low, ambient (unaltered), or high (MMS experiment). Treatments with an * received an addition of Fe.

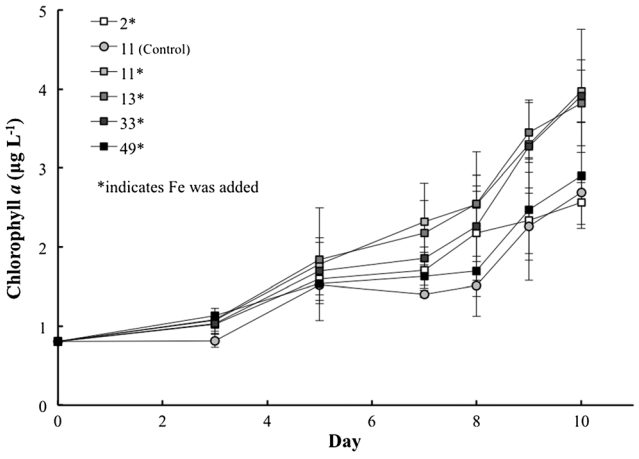


**Figure 1.** Mean chlorophyll *a* concentrations ±1 standard deviation of the whole community (>0.7 µm) over the course of the Terra Nova Bay experiment. Treatment names describe the N:P ratio, and treatments with an * received an addition of Fe.

were present in all treatments at the end of the experiment; hence, changes that we observed for both particulate parameters and uptake rates were not due to relief from a macronutrient limitation.

#### 3.1.1. TNB Microbial Response

The initial concentration of chlorophyll *a* for the whole community (> 0.7 µm) was 0.8 ± 0.1 µg/L (Figure 1). Growth was slow for the first 3 days of the experiment but increased thereafter and remained in near exponential growth until the experiment ended. Treatment chlorophyll *a* concentrations were clustered into two groups, which became evident on Days 9 and 10. The highest chlorophyll *a* concentrations occurred when the N:P ratio was between 11 and 33 and Fe was added. Significantly lower ($p < 0.04$; combined Days 9 and 10 data) concentrations were found in the Control (N:P ratio of 11 with no Fe added) and at the two extremes (N:P ratios of 2 and 49 with Fe added). Most studies from the Southern Ocean show that phytoplankton growth is stimulated with Fe addition (Boyd et al., 2000; Coale et al., 2004; De Baar et al., 1990; Martin et al., 1990); therefore, similarities in chlorophyll *a* concentrations between the Control and the N:P extremes may indicate that even when Fe is added, phytoplankton growth may be negatively impacted when dissolved N:P ratios are outside of an optimal stoichiometric range. Optimal phytoplankton N:P ratios range from approximately 8 to 42 (Klausmeier et al., 2004), so it is possible that when dissolved N:P ratios of an environment are outside of this range, which was the case for the two extreme treatments in this study (N:P ratios of 2 and 49 with Fe added), certain physiological processes, such as production of chlorophyll *a*, are not enhanced even when resources are replete.

#### 3.1.2. TNB Uptake Results.

*DIC*: Absolute DIC uptake rates ($\rho_{DIC}$) by the >5.0-µm size fraction were significantly greater ($p < 0.001$) than the 0.7- to 5.0-µm size fraction for both the initial community and on Day 3 for all treatments (Figure 2a). By Day 10, the opposite pattern occurred (Figure 2b). Within the treatments, Fe addition alone (11*) and a moderate increase (13*) to the N:P ratio resulted in higher ($p < 0.05$) $\rho_{DIC}$ compared to the Control for both size fractions (Figures 2a and 2b). Additionally, on Day 3, all treatments with elevated N:P ratios had higher ($p < 0.01$) $\rho_{DIC}$ than the Control for the >5.0-µm size fraction. For the 0.7- to 5.0-µm size fraction, the 33* treatment was significantly higher ($p < 0.01$) than the Control on Day 10 but not on Day 3.

*AA-C*: For the initial TNB community and the experimental treatments, absolute AA-C uptake rates ($\rho_{AA\text{-}C}$) of the 0.7–5.0 µm size fraction were significantly greater ($p < 0.001$) than the >5.0-µm size fraction with the exception of the Control (Figure 2c). In the 0.7- to 5.0-µm size fraction, the 11* treatment and treatments with elevated N:P ratios had higher ($p < 0.01$) $\rho_{AA\text{-}C}$ than the Control. However, none of the treatments were significantly different from each other.

*$NO_3^-$:* Within the initial community, absolute $NO_3^-$ uptake rates ($\rho_{NO3}$) of the 0.7- to 5.0-µm size fraction were significantly higher ($p < 0.03$) than the >5.0-µm size fraction (Figure 3a). This pattern continued on Day 3 but was only significant ($p < 0.001$) in the Control. By Day 10, $\rho_{NO3}$ was not significantly different between the size fractions. For both size fractions when the N:P ratio was at 33* and 49*, $\rho_{NO3}$ was significantly higher ($p < 0.02$) than the other treatments on Day 3 (Figure 3a). Additionally, within the >5.0-µm size fraction, the 11* and 13* treatments were higher ($p < 0.04$) than the Control on Day 3 (Figure 3a). By Day 10, $\rho_{NO3}$ of all treatments in both size fractions were similar (Figure 3b).

*AA-N*: Absolute AA-N uptake rates ($\rho_{AA\text{-}N}$) had the same pattern as $\rho_{AA\text{-}C}$. For the initial community, $\rho_{AA\text{-}N}$ of the 0.7- to 5.0-µm size fraction was more than triple that of the >5.0-µm size fraction (Figure 3c). Significantly higher $\rho_{AA\text{-}N}$ in the 0.7- to 5.0-µm size fraction also occurred during the experiment ($p < 0.001$), with the exception of the Control.

#### 3.1.3. TNB Uptake Discussion

Although uptake trends varied between substrates as well as over the course of the experiment, several of the findings from this study stand out. When the N:P ratio was decreased to 2, $\rho_{DIC}$ and $\rho_{NO3}$ were not significantly different from the Control in either size fraction at any point during the experiment, indicating that

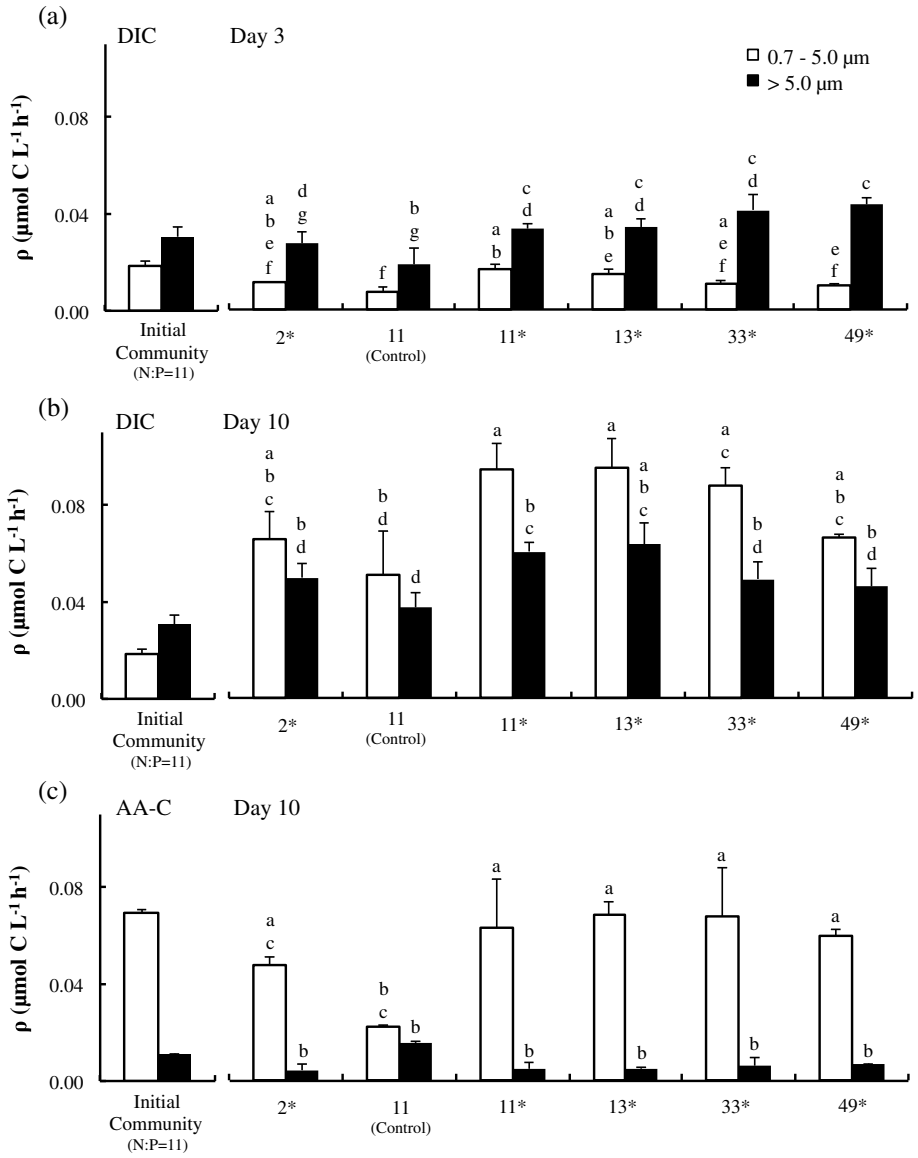


**Figure 2.** Mean absolute C uptake rates ±1 standard deviation of dissolved inorganic carbon (DIC) on Day 3 (a) and Day 10 (b) and of AA-C (c) on Day 10 for the Terra Nova Bay experiment by small (0.7–5.0 µm, white bars) and large (>5.0 µm, black bars) microorganisms. DIC (a and b) and AA-C (c) uptake at the start of the experiment (initial community) is also shown. Bars within a plot that have different letters are significantly different from one another ($p \leq 0.05$). Treatment names describe the N:P ratio, and treatments with an * received an addition of Fe.

even when Southern Ocean microorganisms are relieved of Fe stress, uptake of DIC and $NO_3^-$ may not increase if N:P ratios are low. This was unexpected, because, even though $PO_4^{3-}$ was added to the treatment and proportionately more available than $NO_3^-$, the concentration of $NO_3^-$ was still high (5.74 ± 0.1 µmol N $L^{-1}$) and should therefore have been accessible for enhanced uptake upon Fe addition. This finding merits further research and could indicate that when an environment is characterized by low N:P ratios, DIC and $NO_3^-$ utilization by certain microbial communities may not increase even when Fe stress is eliminated. Another explanation is that the higher concentrations of $PO_4^{3-}$, hence a low N:P ratio, caused the community to exert more energy toward uptake of P rather than uptake of C and N. Although we were not able to measure P uptake in this study, particulate concentrations of P in the 2* treatment were significantly higher than the Control on Day 3 and Day 10 (Table S1), indicating that P was being taken up and assimilated.

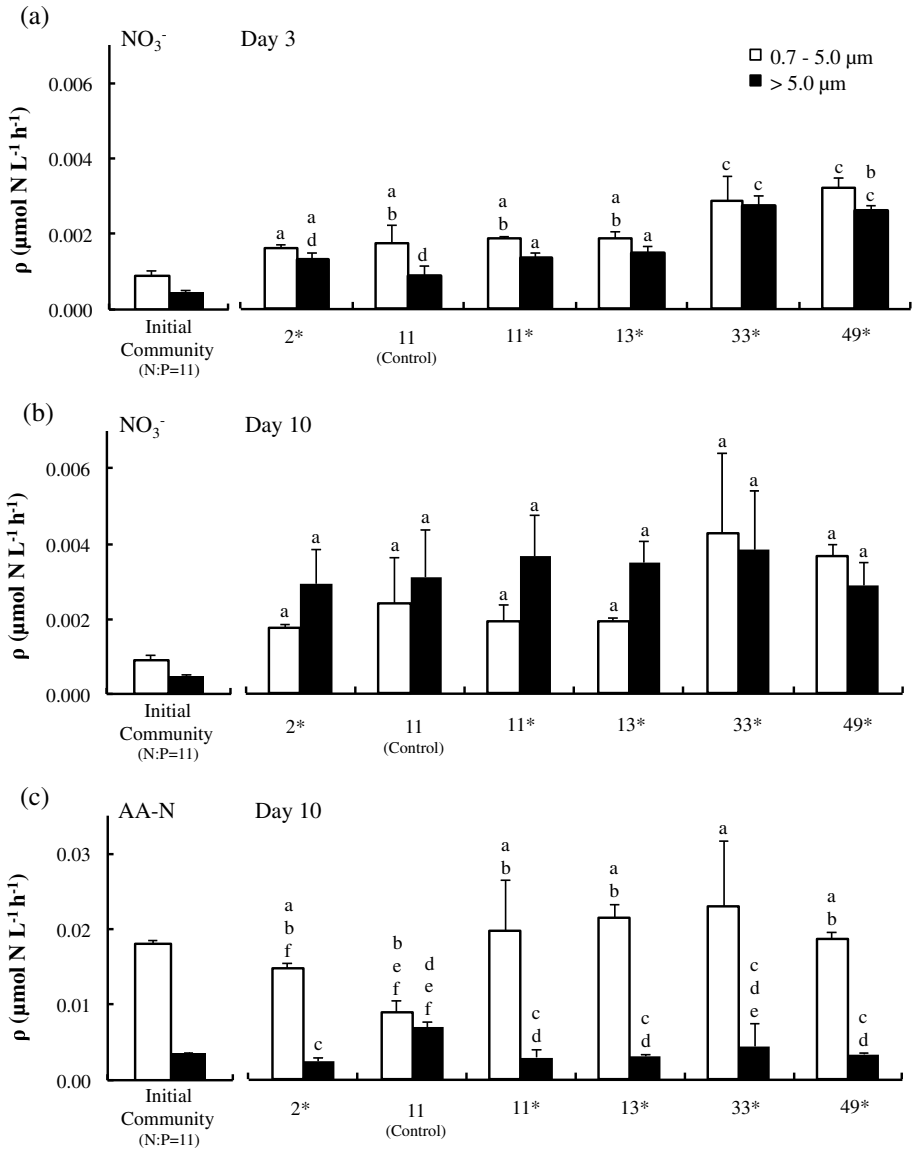


**Figure 3.** Mean absolute N uptake rates ±1 standard deviation of $NO_3^-$ on Day 3 (a) and Day 10 (b) and of AA-N (c) on Day 10 for the Terra Nova Bay experiment by small (0.7–5.0 µm, white bars) and large (>5.0 µm, black bars) microorganisms. $NO_3^-$ (a and b) and AA-N (c) uptake at the start of the experiment (initial community) is also shown. Bars within a plot that have different letters are significantly different from one another ($p \leq 0.05$). Treatment names describe the N:P ratio, and treatments with an * received an addition of Fe.

Our data also suggest that changes to dissolved stoichiometric N:P ratios could alter uptake rates of $NO_3^-$ over short time scales, but that Southern Ocean microorganisms may display physiological plasticity (i.e., ability to acclimate to environmental change) to help them adapt to stoichiometric changes. In treatments where $\rho_{NO3}$ significantly increased, the change occurred at the beginning of the experiment (Figure 3a). Fe addition may have been the main factor influencing change on Day 3; however, $\rho_{NO3}$ for both size fractions in treatments where the N:P ratio was ≥33 were significantly higher than the treatment that received only Fe (11*). This could indicate that there is an additive effect of elevated N:P and Fe on $NO_3^-$ uptake in HNLC regions. Luxury consumption of $NO_3^-$ is known to occur when $NO_3^-$ is replete (Lomas & Glibert, 2000; Rhee, 1978) and may have occurred during the initial stages of the experiment (Figure 3a) and decelerated by the end of the experiment (Figure 3b). This could explain why there were no significant differences in $\rho_{NO3}$ between treatments on Day 10 (Figure 3b).

**Table 3**
*Particulate Ratios of the Initial Communities and the Treatment Communities*

| | | C:N | N:P | C:P |
|---|---|---|---|---|
| TNB Site | | | | |
| | | 6.3 ± 0.2 | 16.1 ± 0.5 | 102 ± 5.3 |
| TNB (Day 3) | | | | |
| | 2* | 8.2 ± 0.3 | 14.8 ± 0.4[a] | 122 ± 2.1[a] |
| | 11 (Control) | 8.3 ± 0.3 | 25.5 ± 4.0[a] | 212 ± 41 |
| | 11* | 8.1 ± 0.3 | 23.1 ± 6.1 | 187 ± 49 |
| | 13* | 7.9 ± 0.2 | 15.7 ± 0.7[a] | 123 ± 3.2[a] |
| | 33* | 8.0 ± 0.1 | 15.7 ± 1.7[a] | 125 ± 14[a] |
| | 49* | 8.0 ± 0.7 | 15.7 ± 1.0[a] | 126 ± 20[a] |
| TNB (Day 10) | | | | |
| | 2* | 6.6 ± 0.3 | 12.0 ± 0.6 | 79.2 ± 7.1 |
| | 11 (Control) | 6.7 ± 0.0 | 13.1 ± 0.5 | 87.3 ± 3.4 |
| | 11* | 6.4 ± 0.1 | 13.5 ± 0.3 | 86.4 ± 2.4 |
| | 13* | 6.4 ± 0.1 | 14.9 ± 0.1[a] | 95.2 ± 1.6 |
| | 33* | 6.5 ± 0.1 | 12.5 ± 0.7 | 81.0 ± 4.7 |
| | 49* | 6.4 ± 0.1 | 14.0 ± 0.4 | 90.2 ± 3.1 |
| MMS Site | | | | |
| | | 7.2 ± 0.5 | 12.8 ± 0.5 | 92.9 ± 7.3 |
| MMS (Day 7) | | | | |
| 0 °C Treatments | Low | 7.9 ± 0.2 | 16.7 ± 0.7 | 132 ± 3.0 |
| | Ambient (Control) | 8.0 ± 0.2 | 16.9 ± 0.8 | 136 ± 5.9 |
| | High | 8.0 ± 0.0 | 16.4 ± 1.6 | 132 ± 12 |
| | Low* | 7.3 ± 0.1[a] | 16.2 ± 0.8 | 118 ± 6.2 |
| | Ambient* | 7.3 ± 0.0[a] | 18.6 ± 0.5 | 136 ± 4.1 |
| | High* | 7.5 ± 0.1[a] | 16.5 ± 0.9 | 124 ± 4.9 |
| 4 °C Treatments | Low | 8.0 ± 0.2 | 18.9 ± 2.4 | 150 ± 17 |
| | Ambient | 8.1 ± 0.2 | 20.9 ± 7.8 | 170 ± 67 |
| | High | 8.4 ± 0.1 | 21.7 ± 4.2 | 181 ± 34 |
| | Low* | 7.7 ± 0.0 | 18.2 ± 0.2 | 140 ± 0.7 |
| | Ambient* | 7.7 ± 0.1 | 19.7 ± 0.9 | 152 ± 8.4 |
| | High* | 7.8 ± 0.1 | 18.8 ± 1.6 | 146 ± 11 |

*Note.* Mean particulate ratios ±1 standard deviation ($n = 3$) of the community (>0.7 μm size fraction) collected from each site (TNB and MMS), and mean particulate ratios of the communities from each treatment measured on Day 3 and Day 10 for TNB, and on Day 7 for MMS.
[a]Particulate ratios are significantly different from the control ($p \leq 0.05$) on that particular day. Treatment names describe either the N:P ratio (Terra Nova Bay, TNB, experiment) or whether the N:P ratio was low, ambient, or high (McMurdo Sound, MMS, experiment). Treatments with an * received an addition of Fe.

Finally, the high $\rho_{AA\text{-}C}$ and $\rho_{AA\text{-}N}$ that were measured, particularly in the smaller size fraction, indicate that Fe addition resulted in smaller microorganisms having a competitive edge over larger microorganisms in their ability to take up amino acids (Figures 2c and 3c). The considerable amount of uptake by the smaller size fraction is likely attributable to bacteria, as the majority of bacteria (88.8%) at our site were retained on the GFF filter. Higher $\rho_{AA\text{-}C}$ and $\rho_{AA\text{-}N}$ by smaller microorganisms compared to larger microorganisms is the same trend that was observed for $\rho_{DIC}$ on Day 10 and makes sense given that bacterial abundance increased at the end of the experiment (supporting information Figure S1). Changes to nutrient ratios may have also influenced amino acid uptake rates, yet far less noticeably than Fe addition. In the smaller size fraction, statistically significant differences were observed for $\rho_{AA\text{-}C}$ when only Fe was added or when N:P ratios were increased, and for $\rho_{AA\text{-}N}$ significant differences occurred when the N:P ratio was at 13 and 33. When the ratio was decreased to 2, $\rho_{AA\text{-}C}$ and $\rho_{AA\text{-}N}$ were similar to the control, and in the larger size fraction $\rho_{AA\text{-}N}$ was significantly lower. These findings further support the idea that uptake rates of both inorganic and organic C and N substrates may not be enhanced in HNLC regions when N:P ratios are considerably low, even when Fe is added. Studies of amino acid utilization by microorganisms have primarily been conducted in oligotrophic regions such as the Sargasso Sea (Jørgensen et al., 1993; Keil & Kirchman, 1999; Suttle et al., 1991; Zubkov et al., 2008), and the small amount of research done in the Southern Ocean show that turnover of amino acids is high (Simon & Rosenstock, 2007) and that utilization is influenced by irradiance

**Table 4**
*Abundance and Characteristics of Phaeocystis Colonies*

| | | Colonies ($ml^{-1}$) | Cells ($colony^{-1}$) | Relative abundance (%) |
|---|---|---|---|---|
| 0 °C | Low | 137 ± 94 | 35 ± 13 | 93.2 |
| treatments | Ambient (Control) | 110 ± 33 | 29 ± 10 | 84.3 |
| | High | 107 ± 2.0 | 47 ± 14 | 88.3 |
| | Low* | 59 ± 30 | 42 ± 26 | 89.3 |
| | Ambient* | 95 ± 18 | 19 ± 13 | 76.4 |
| | High* | 122 ± 65 | 36 ± 18 | 81.3 |
| 4 °C | Low | 167 ± 42 | 17 ± 1.7 | 77.7 |
| treatments | Ambient | 164 ± 74 | 22 ± 1.9 | 85.5 |
| | High | 230 ± 50 | 24 ± 1.9 | 90.4 |
| | Low* | 700 ± 69 | 22 ± 17 | 87.6 |
| | Ambient* | 507 ± 12 | 22 ± 5.6 | 87.5 |
| | High* | 413 ± 151 | 20 ± 7.7 | 89.4 |

*Note*. Mean *Phaeocystis* colonies ±1 standard deviation ($n = 3$), mean number of *Phaeocystis* cells per colony ±1 standard deviation ($n = 3$), and the relative abundance (% of all phytoplankton cells) of colonial *Phaeocystis* cells comprising the final phytoplankton community of each treatment. Treatment names describe whether the N:P ratio was low, ambient, or high, and treatments with * received an addition of Fe.

(Rivkin & Putt, 1987). Our data indicate that more research is needed in HNLC regions to examine the effects of Fe addition on dissolved organic nutrient utilization, because of its potential for shaping biogeochemical cycles, particularly with respect to phytoplankton-bacterial interactions.

#### 3.1.4. TNB Cellular Stoichiometry

Compared to the ratios measured at the TNB site, particulate C:N and C:P ratios increased on Day 3 for all treatments (Table 3), likely because concentrations of particulate C increased (Table S1). By Day 10, however, concentrations of particulate C had decreased compared to Day 3, and particulate C:N ratios were similar to the starting ratio, while C:P ratios were lower than the starting ratio. From Day 3 to Day 10, particulate N:P and C:P ratios of all treatments decreased. This trend may have been a result of the communities using and allocating proportionately more P within their cells toward growth machinery, such as ribosomal RNA. Concentrations of particulate P increased in all treatments from Day 3 to Day 10, which supports this idea (Table S1).

Particulate C:N ratios did not differ among the treatments within a given time point. Significant differences were identified, however, for particulate N:P and C:P ratios on Day 3, where the Control had higher ($p < 0.05$) particulate N:P and C:P ratios than all of the other treatments except for the 11* treatment. This difference was primarily driven by relatively lower concentrations of particulate P in the Control and the 11* treatment (supporting information Table S1). By Day 10, particulate N:P and C:P ratios of the treatments were generally similar to one another. The lowest particulate N:P and C:P ratios were found in the 2* treatment and highest ratios in the 13* treatment, and the difference between these two treatments for both particulate N:P and C:P was significant ($p < 0.001$). Larger cellular stoichiometric changes occurred on Day 3 in comparison to Day 10, further supporting the idea that the impacts associated with changing nutrient supply ratios in the Southern Ocean may happen on the order of a few days and that microorganisms display physiological plasticity.

The results from the TNB experiment and a series of parallel studies (Bertrand et al., 2015; Zhu et al., 2016; Spackeen et al., 2018) allowed us to reassess our experimental approach for the MMS experiment (results below). Parallel studies provided important evidence that temperature may play a key role in altering physiological rates and thus may affect how organisms respond to alterations in N:P ratios. For the MMS study, we were able to optimize our efforts by reducing the number of N:P ratios and by adding a more in depth investigation into how Fe and temperature affect the stoichiometric response of Antarctic microbial communities.

### 3.2. MMS Experiment

The microbial assemblage used in the MMS experiment was collected during an earlier phase of the Ross Sea phytoplankton bloom than the TNB experiment. Concentrations of $NO_3^-$, $PO_4^{3-}$, and Si at the MMS site were more than double the concentrations measured at the TNB site, while concentrations of $NH_4^+$ and organic forms of N (urea and DPA) were lower (Table 2). These differences are likely due to peak-season (MMS) versus late season (TNB) sampling. The $NO_3^-$:$PO_4^{3-}$ ratio of the MMS site water was ~14.9, and ratios of the experimental treatments remained within ~3 units of the target ratios during the experiment (Tables 1 and 2). By the end of the MMS experiment, concentrations of $NO_3^-$ had changed the most, decreasing by more than 7.5 μmol N $L^{-1}$, in treatments at 4 °C that were Fe replete. During the experiment, diatoms were not a major component of the microbial assemblage (Table 4) and concentrations of Si did not change (Table 2).

#### 3.2.1. MMS Microbial Response

The initial concentration of chlorophyll *a* for the whole MMS community (> 0.7 μm) was 2.4 ± 0.2 μg/L (Figure 4). During the experiment, concentrations of chlorophyll *a* steadily increased from Day 3 until the end of the experiment. All treatments at 0 °C had similar chlorophyll *a* concentrations (Figure 4a). The treatments at 4 °C were separated into two groups; treatments with Fe had the highest chlorophyll *a* concentrations, and treatments without Fe had similar concentrations to the 0 °C treatments (Figure 4b). A similar result also occurred with particulate concentrations, where treatments at 4 °C with added Fe had significantly higher particulate C and N concentrations than the Control ($p < 0.001$; Table S1). The lack of response to

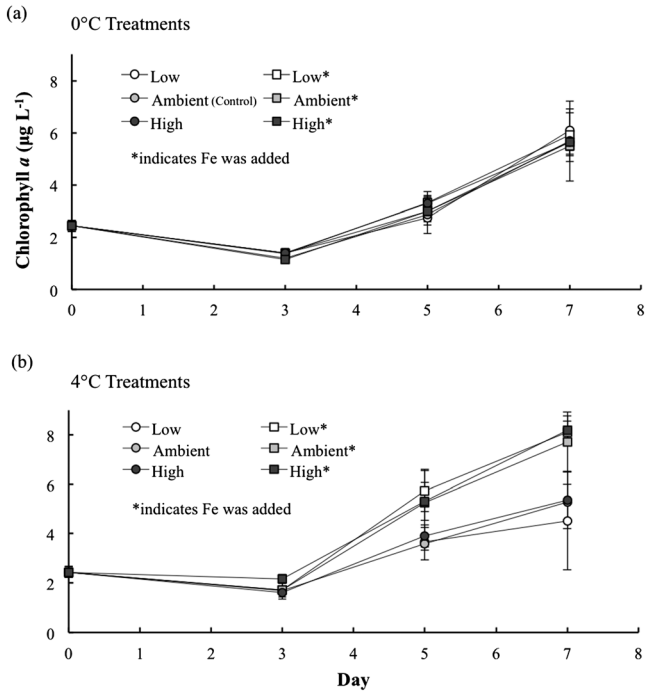


**Figure 4.** Mean chlorophyll *a* concentrations ±1 standard deviation of the whole community (>0.7 µm) at 0 °C (a) and 4 °C (b) over the course of the McMurdo Sound experiment. Treatment names describe whether the N:P ratio was low, ambient, or high, and treatments with an * received an addition of Fe.

Fe addition at 0 °C indicates that temperature elevation may be necessary to drive an Fe addition response by certain Ross Sea microbial communities. The additive effect of temperature elevation and Fe addition on increasing chlorophyll *a* concentrations of Ross Sea phytoplankton has previously been shown (Rose et al., 2009).

### 3.2.2. MMS Uptake Results.

*DIC*: The initial MMS community had the same pattern as the TNB community in that the >5-µm size fraction had higher $\rho_{DIC}$ ($p < 0.001$) than the 0.7- to 5.0-µm size fraction (Figure 5a). This pattern also occurred during the experiment ($p < 0.001$), with the exception of the 4 °C − Fe set of treatments where $\rho_{DIC}$ of the two size fractions were not significantly different (Figure 5a). Comparing the treatments to the Control, $\rho_{DIC}$ increased ($p < 0.001$) for the >5.0-µm size fraction when Fe was added, and $\rho_{DIC}$ increased ($p < 0.01$) for the 0.7- to 5.0-µm size fraction at 4 °C. In the 0.7- to 5.0-µm size fraction, $\rho_{DIC}$ also increased ($p < 0.01$) in the 0 °C ambient* treatment (unaltered N:P ratio with added Fe).

*AA-C*: For the initial community, $\rho_{AA\text{-}C}$ of the 0.7- to 5.0-µm size fraction was significantly greater ($p < 0.001$) than the >5.0-µm size fraction (Figure 5b). Highest $\rho_{AA\text{-}C}$ for both size fractions occurred in the 4 °C + Fe set of treatments (Figure 5b). In the >5.0-µm size fraction, Low* and High* N:P ratios at 4 °C resulted in higher ($p < 0.001$; * indicates Fe was added) $\rho_{AA\text{-}C}$ than the Control and the 0 °C Low and 0 °C ambient* treatments.

$NO_3^-$: The >5.0 µm size fraction had higher $\rho_{NO3}$ than the 0.7- to 5.0-µm size fraction for the 0 °C − Fe, 0 °C + Fe, and 4 °C + Fe set of treatments (Figure 6a). The opposite pattern occurred in the 4 °C − Fe set of treatments (Figure 6a). Taking a closer look at the individual size fractions,

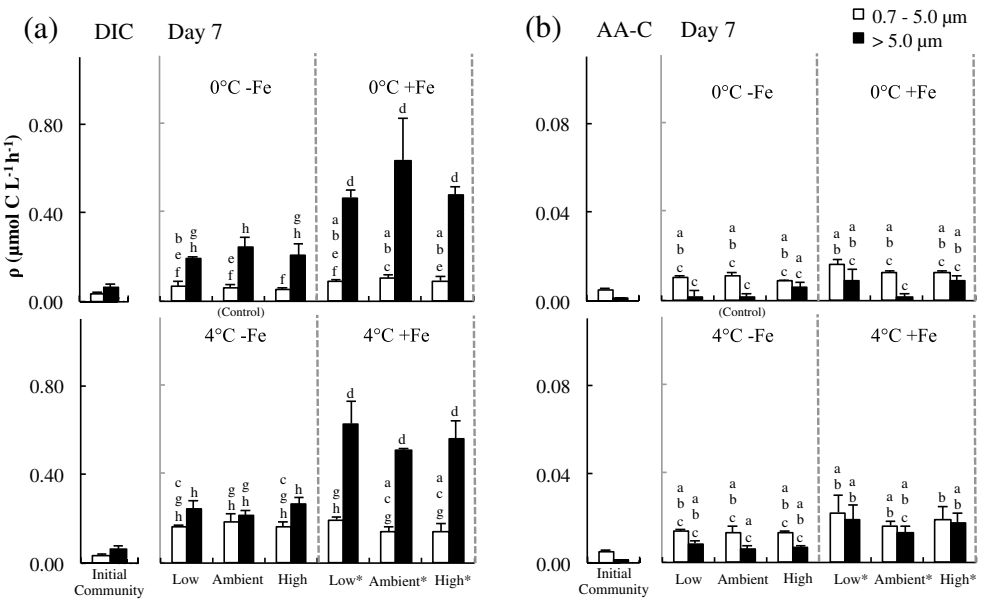


**Figure 5.** Mean absolute C uptake rates ±1 standard deviation of dissolved inorganic carbon (DIC) (a) and AA-C (b) for the MMS experiment by small (0.7–5.0 µm, white bars) and large (>5.0 µm, black bars) microorganisms on Day 7 of the experiment. DIC (a) and AA-C (b) uptake at the start of the experiment (initial community) is also shown. Bars that have different letters are significantly different from one another ($p \leq 0.05$); all data for treatments within a single panel (i.e., a or b) were included in the comparison. Treatment names describe whether the N:P ratio was low, ambient, or high, and treatments with an * received an addition of Fe.

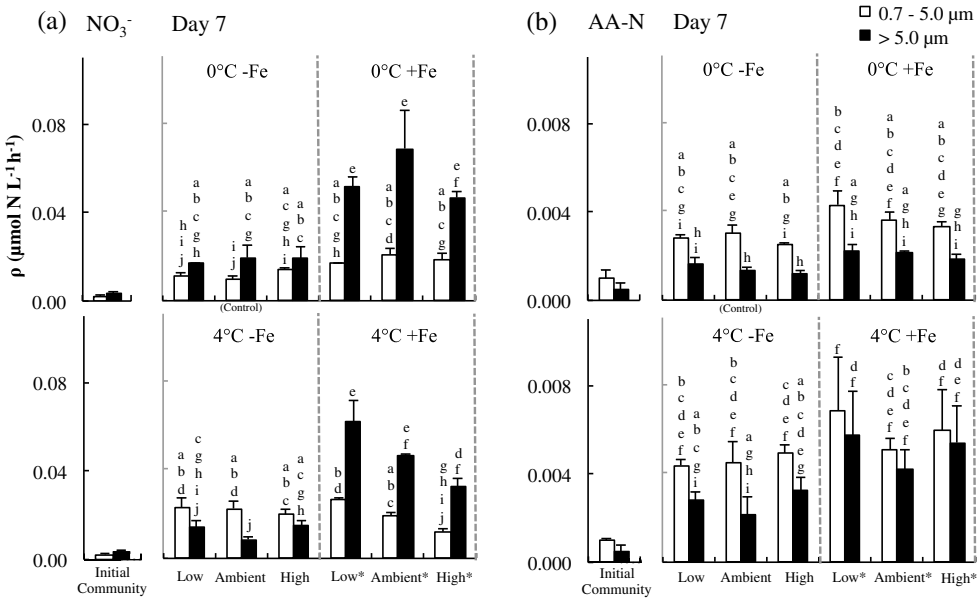


**Figure 6.** Mean absolute N uptake rates ±1 standard deviation of $NO_3^-$ (a) and AA-N (b) for the McMurdo Sound experiment by small (0.7–5.0 µm, white bars) and large (>5.0 µm, black bars) microorganisms. $NO_3^-$ (a) and AA-N (b) uptake at the start of the experiment (initial community) is also shown. Bars that have different letters are significantly different from one another ($p \leq 0.05$); all data for treatments within a single panel (i.e., a or b) were included in the comparison. Treatment names describe whether the N:P ratio was low, ambient, or high, and treatments with an * received an addition of Fe.

$\rho_{NO3}$ in the >5.0-µm size fraction had the same pattern as $\rho_{DIC}$, where all Fe-replete treatments had higher ($p < 0.01$) $\rho_{NO3}$ than the Control. In the 0.7- to 5.0-µm size fraction, all treatments at 4 °C, or where Fe was replete, had higher ($p < 0.001$) $\rho_{NO3}$ than the Control, with the exception of the 4 °C High* treatment.

*AA-N*: Following the same pattern as $\rho_{AA\text{-}C}$, the highest $\rho_{AA\text{-}N}$ also occurred in the 4 °C + Fe set of treatments (Figure 6b). In the >5.0-µm size fraction, the 4 °C + Fe set of treatments, with the exception of 4 °C Ambient*, were significantly higher ($p < 0.03$) than all treatments set at 0 °C in addition to the 4 °C Low and Ambient treatments. In the 0.7- to 5.0-µm size fraction, the 4 °C + Fe set of treatments, with the exception of the 4 °C Ambient* treatment, had higher ($p < 0.02$) $\rho_{AA\text{-}N}$ than the 0 °C − Fe set of treatments.

### 3.2.3. MMS Uptake Discussion

During this experiment, temperature and/or Fe addition impacted uptake rates by the microbial community, while the effects of changing the dissolved N:P ratio were less pronounced. The observation that Fe addition increased $\rho_{DIC}$ and $\rho_{NO3}$ (Figures 5a and 6a) is consistent with previous experiments, which typically show that Fe addition increases nutrient uptake by Southern Ocean microorganisms (Cochlan, 2008, and references therein; Bertrand et al., 2015; Hutchins & Boyd, 2016). For the larger size fraction, Fe addition rather than temperature was responsible for higher $\rho_{DIC}$ and $\rho_{NO3}$. This is supported by a lack of response in the 4 °C − Fe set of treatments and the absence of an additive effect in the 4 °C + Fe set of treatments. In this study the microbial community was dominated by colonial *Phaeocystis* cells (discussed below); thus, our uptake results for the larger size fraction indicate that Fe addition rather than temperature elevation have a larger influence on increasing nutrient uptake rates by *Phaeocystis*. Studies show that other prominent Southern Ocean microorganisms may have a competitive advantage over *Phaeocystis* in a warmer Southern Ocean (Boyd et al., 2016; Coello-Camba & Agusti, 2017; Petrou et al., 2016; Zhu et al., 2016) and that *Phaeocystis* growth can decline at higher temperatures (Xu et al., 2014). Our results suggest that as long as Fe is present, nutrient utilization by *Phaeocystis* will likely increase, placing this taxa in a position to better compete with other dominant phytoplankton in a warmer Southern Ocean.

In the smaller size fraction, with the exception of a few treatments, $\rho_{DIC}$ and $\rho_{NO3}$ were higher as a function of both temperature and Fe, individually and in combination. Even though Fe was not added to the 4 °C − Fe set of treatments, both $\rho_{DIC}$ and $\rho_{NO3}$ of the smaller size fraction were higher than the Control. This could be due to bacterial uptake of $NO_3^-$. Bacterial abundance was higher in these treatments than the Control

(supporting information Figure S2). Temperature is known to enhance microbial metabolism (Delille, 2004; Hutchins & Fu, 2017; Pomeroy & Wiebe, 2001), and research has demonstrated that elevated temperature may be equally as important as Fe limitation on impacting nutrient uptake by Southern Ocean microorganisms (Hutchins & Boyd, 2016; Reay et al., 2001). It is also possible that in the 4 °C − Fe set of treatments, the smaller size fraction may have been able to access any available Fe more efficiently than the larger size fraction due to their smaller surface area to volume ratios. This could have implications for C and N cycling in a warmer Southern Ocean when Fe is not readily available.

Comparing trends that occurred for concentrations of chlorophyll *a* with trends that were observed for $\rho_{DIC}$ and $\rho_{NO3}$ indicate that the microorganisms may allocate C and N toward different resources when they are at 0 °C versus 4 °C and Fe is added. Concentrations of chlorophyll *a* only increased when both the temperature was elevated and Fe was added; however, $\rho_{DIC}$ and $\rho_{NO3}$ increased at both 0 °C and 4 °C when Fe was added. It is possible that the microorganisms, namely, *Phaeocystis*, in this experiment may have been using the C and N that they were taking up upon Fe addition at 0 °C toward the synthesis of proteins other than chlorophyll *a* and that temperature elevation combined with Fe addition allowed them to allocate more C and N toward production of chlorophyll *a*.

In the MMS experiment, $\rho_{AA\text{-}C}$ and $\rho_{AA\text{-}N}$ were not nearly as high as those measured during the TNB experiment, particularly for smaller microorganisms. This is likely due to differences between the communities and overall lower bacterial abundance in the MMS experiment (supporting information Figures S1 and S2). Community differences may also explain why trends between the two experiments differed. For the TNB experiment, Fe addition likely impacted amino acid utilization by smaller microorganisms (Figures 2c and 4c), while in the MMS experiment increasing Fe and temperature in combination were needed to produce the highest $\rho_{AA\text{-}C}$ and $\rho_{AA\text{-}N}$. This was evident, significantly in some cases, for both small and large microorganisms (Figures 5b and 6b). Based on the findings from both the TNB and MMS experiment, climate change parameters affect microbial utilization of amino acids, and perhaps other organic substrates, and are a topic that should be further investigated.

Changes in uptake rates due to changing the dissolved N:P ratio were limited to the smaller size fraction, and trends were less clear than those found in the TNB experiment. Within the 0 °C + Fe set of treatments, $\rho_{DIC}$ increased when Fe alone was added but did not increase when nutrient ratios were changed in either direction. Within the 4 °C + Fe set of treatments, $\rho_{NO3}$ increased in both the 4 °C Low* and Ambient* treatments, but rates were not impacted in the 4 °C High* treatment. Although the influence of N:P ratios was clearer in the TNB experiment than the MMS experiment, a few inferences can be made. In the MMS experiment, any impact on $NO_3^-$ uptake rates due to increasing the N:P ratio may have occurred prior to the Day 7 assessments. This is supported by $NO_3^-$ concentrations in the high N:P treatments, which decreased the most during the first few days of the experiment (supporting information Table S2), and that most changes due to stoichiometry occurred on Day 3 rather than Day 10 in the TNB experiment. When the N:P ratio was low in combination with Fe addition, rates were unaffected in the TNB experiment, yet increased in the MMS experiment. This may indicate that there is a N:P threshold at which growth and nutrient uptake is not enhanced regardless of whether Fe is present, or that microorganisms that bloom toward the end of the austral summer are more sensitive to low N:P ratios than those that bloom earlier in the season.

#### 3.2.4. MMS Cellular Stoichiometry

Compared to the ratios of the starting community, particulate C:P and N:P ratios of all of the treatments increased over the course of the MMS experiment, while C:N ratios remained similar to the initial ratio (Table 3). The increase in particulate C:P and N:P ratios occurred because concentrations of particulate C and N increased relatively more than concentrations of particulate P (Table S1). The relative increase in C:P and N:P ratios from the start to the end of the experiment likely occurred due to the dominance of *Phaeocystis* (discussed below), which may have been allocating C and N toward the formation of colony matrices.

By Day 7 of the experiment, cellular particulate ratios were mostly unaffected, with the exception of C:N ratios in the treatments that were at 0 °C and Fe replete, which were significantly lower than most of the other treatments. The 0 °C Low* and 0 °C Ambient* treatments were lower ($p < 0.05$) than all other treatments that were either incubated at 4 °C or at 0 °C without Fe, and the 0 °C High* treatment was lower ($p < 0.04$) than all treatments that were Fe deplete. For particulate N:P and C:P ratios, there were no significant differences identified

**Table 5**
*Absolute and Relative Abundance of Phytoplankton Taxa for Each Treatment on the Final Day of the MMS Experiment*

| | | *Phaeocystis* (colonial cells) | *Phaeocystis* (solitary cells) | Dinoflagellates | *Pseudo-nitzschia* spp. | *Fragilariopsis* spp. | Other Pennates | Centrics | Other |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Absolute abundance (cells/ml) | | | | | |
| 0 °C | Low | 5,384 ± 3,945 | 105 ± 30 | 183 ± 55 | 8.0 ± 4 | 50.7 ± 27 | 0.0 ± 0.0 | 24.0 ± 31 | 20.0 ± 14 |
| Treatments | Ambient (Control) | 2,983 ± 825 | 212 ± 30 | 278 ± 23 | 10.0 ± 5 | 26.7 ± 15 | 8.3 ± 14 | 6.7 ± 5.8 | 13.3 ± 8 |
| | High | 3579 ± 1157 | 121 ± 22 | 219 ± 41 | 31.5 ± 23 | 24.5 ± 28 | 0.0 ± 0.0 | 33.5 ± 2.1 | 46.5 ± 26 |
| | Low* | 2,649 ± 2,395 | 47.2 ± 13 | 153 ± 24 | 0.0 ± 0.0 | 75.6 ± 119 | 15.6 ± 4 | 17.8 ± 31 | 7.8 ± 2 |
| | Ambient* | 1,787 ± 1,095 | 243 ± 124 | 237 ± 60 | 13.3 ± 10 | 15.0 ± 8.7 | 0.0 ± 0.0 | 8.3 ± 3 | 35.0 ± 15 |
| | High* | 3,373 ± 800 | 198 ± 241 | 473 ± 249 | 13.3 ± 23 | 15.6 ± 7.7 | 0.0 ± 0.0 | 28.9 ± 14 | 48.9 ± 28 |
| 4 °C | Low | 2,793 ± 603 | 447 ± 74 | 280 ± 46 | 36.7 ± 23 | 10.0 ± 17 | 16.7 ± 29 | 3.3 ± 6 | 10.0 ± 10 |
| Treatments | Ambient | 3,598 ± 1,847 | 222 ± 130 | 324 ± 235 | 28.9 ± 33 | 2.2 ± 4 | 0.0 ± 0.0 | 24.4 ± 27 | 8.9 ± 10 |
| | High | 5,640 ± 1,621 | 220 ± 61 | 270 ± 61 | 73.3 ± 59 | 0.0 ± 0.0 | 13.3 ± 23 | 13.3 ± 15 | 6.7 ± 6 |
| | Low* | 15,347 ± 13215 | 1,333 ± 410 | 667 ± 151 | 60.0 ± 40 | 75.7 ± 20 | 20.0 ± 20 | 20.0 ± 35 | 20.0 ± 20 |
| | Ambient* | 11,200 ± 2620 | 880 ± 246 | 523 ± 60 | 53.3 ± 23 | 35.1 ± 17 | 16.7 ± 29 | 23.3 ± 25 | 20.0 ± 0.0 |
| | High* | 8,093 ± 4072 | 487 ± 250 | 300 ± 122 | 113 ± 50 | 23.1 ± 20 | 20.0 ± 0.0 | 26.7 ± 12 | 0.0 ± 0.0 |
| | | | | Relative abundance (%) | | | | | |
| 0 °C | Low | 93.2 | 1.8 | 3.2 | 0.1 | 0.9 | 0.0 | 0.4 | 5.1 |
| Treatments | Ambient (Control) | 84.3 | 6.0 | 7.9 | 0.3 | 0.8 | 0.2 | 0.2 | 2.4 |
| | High | 88.3 | 3.0 | 5.4 | 0.8 | 0.6 | 0.0 | 0.8 | 9.8 |
| | Low* | 89.3 | 1.6 | 5.2 | 0.0 | 2.5 | 0.5 | 0.6 | 2.5 |
| | Ambient* | 76.4 | 10.4 | 10.1 | 0.6 | 0.6 | 0.0 | 0.4 | 6.3 |
| | High* | 81.3 | 4.8 | 11.4 | 0.3 | 0.4 | 0.0 | 0.7 | 6.3 |
| 4 °C | Low | 77.7 | 12.4 | 7.8 | 1.0 | 0.3 | 0.5 | 0.1 | |
| Treatments | Ambient | 85.5 | 5.3 | 7.7 | 0.7 | 0.1 | 0.0 | 0.6 | 1.2 |
| | High | 90.4 | 3.5 | 4.3 | 1.2 | 0.0 | 0.2 | 0.2 | 1.5 |
| | Low* | 87.6 | 7.6 | 3.8 | 0.3 | 0.3 | 0.1 | 0.1 | 1.1 |
| | Ambient* | 87.5 | 6.9 | 4.1 | 0.4 | 0.6 | 0.1 | 0.2 | 0.9 |
| | High* | 89.4 | 5.4 | 3.3 | 1.3 | 0.1 | 0.2 | 0.3 | 1.3 |

*Note*. Mean absolute abundances ±1 standard deviation (cells/ml; $n = 3$) and relative abundances (% of all phytoplankton cells; $n = 3$) of taxa comprising the final phytoplankton community of each treatment. Treatment names describe whether the N:P ratio was low, ambient, or high, and treatments with * received an addition of Fe. MMS = McMurdo Sound.

among treatments. The impact of elevated Fe and other micronutrients on cellular stoichiometry is not clear, but our results were unexpected given that particulate C:N ratios are typically more stable in nature than N:P and C:P ratios (Martiny et al., 2013; Moore et al., 2013).

#### 3.2.5. MMS Phytoplankton Community Composition

The dominant member of the MMS phytoplankton community was *Phaeocystis antarctica*, a haptophyte that can exist in both solitary and colonial forms (Schoemann et al., 2005). Colonial *Phaeocystis* cells were more abundant than solitary cells at the end of the experiment, and *Phaeocystis* colonial cells comprised 76.4% to 93.2% of the whole phytoplankton treatment communities (Table 4). Although variability was high, most treatments had more than 100 colonies per millimeter. There were generally more cells per colony at 0 °C than at 4 °C, yet there were more colonies per millimeter in the 4 °C treatments than the 0 °C treatments. Abundances of colonies per millimeter were highest in the 4 °C + Fe set of treatments (Table 4). Increases in colony abundance as a function of both a temperature elevation of 4 °C (Wang et al., 2010) and Fe addition (Feng et al., 2010) have previously been observed, so it follows that a 4 °C temperature increase in combination with Fe addition would yield the highest abundance of colonies. The high abundance of *Phaeocystis* colonies in the 4 °C + Fe set of treatments likely explains why concentrations of chlorophyll *a* and particulate C and N were highest in these treatments (Figure 5 and Table S1).

Although variability was high, abundances of phytoplankton groups other than colonial *Phaeocystis* show subtle differences in the distribution of groups/species among treatments (Table 5). Solitary *Phaeocystis* cells and dinoflagellates were relatively abundant in all treatments, with highest abundances (cells/ml) occurring in the 4 °C + Fe set of treatments. In the 0 °C − Fe, 0 °C + Fe, and 4 °C − Fe set of treatments, dinoflagellates were more abundant than solitary *Phaeocystis* cells, with the exception of the 0 °C Ambient* and 4 °C Low treatments. In the 4 °C + Fe set of treatments, the opposite pattern was observed, and solitary *Phaeocystis* cells were relatively more abundant than dinoflagellates. This result suggests that Southern Ocean

dinoflagellates may be at a competitive disadvantage in a warmer Southern Ocean when Fe is present, especially when *Phaeocystis* is dominant. Diatoms, including *Pseudo-nitzschia* spp., *Fragilariopsis* spp., other pennates, and centrics, comprised a small portion of the overall community. For *Pseudo-nitzschia* spp., both absolute and relative abundances were highest when the temperature was at 4 °C and when the N:P ratio was high, regardless of whether Fe was replete or deplete. Growth rates of polar *Pseudo-nitzschia* sp. are known to increase as a function of temperature and/or Fe (Boyd et al., 2016; Zhu et al., 2016), and the results from this study indicate that *Pseudo-nitzchia* may have a competitive advantage when the N:P supply ratio is high. Although *Fragilariopsis* spp. had a higher absolute abundance in the 4 °C treatments, in the 0 °C Low* treatment *Fragilariopsis* spp. comprised 2.5% of the whole community, which was twofold more than its relative abundance in any of the other treatments. The preferential drawdown of $PO_4^{3-}$ over $NO_3^-$ resulting in higher N:P ratios was attributed to the dominance of *Fragilariopsis kerguelensis* during blooms in the Polar Front (De Baar et al., 1997). Perhaps Fe-replete conditions coupled with proportionately higher concentrations of $PO_4^{3-}$ are favorable for *Fragilariopsis* spp. In all treatments, other pennate diatom species and centric diatoms comprised less than 1% of the whole community, and all other phytoplankton taxa were less than 1.5% of the whole community.

Many studies have assessed the individual and combined effects of global change variables, including temperature elevation (Feng et al., 2009; Hare et al., 2007; Rose et al., 2009), Fe addition (Feng et al., 2010; Rose et al., 2009), and nutrient ratios (Czerny et al., 2016; De Senerpont Domis et al., 2014; Lagus et al., 2004) on community composition. It is clear that global change results in community shifts, and research continues to demonstrate the importance of evaluating the interactive effects of multiple variables to fully understand the microbial community response. In this study, colonial *Phaeocystis* was dominant in all treatments, which was expected considering that *Phaeocystis* usually reaches maximum abundance in the Ross Sea in middle to late December (Smith et al., 2000), the time frame when water was collected for the experiment. Dominance of colonial *Phaeocystis* cells was likely driving nutrient uptake trends, particularly within the larger size fraction, where Fe-replete conditions increased DIC and $NO_3^-$ uptake. Differences among the final communities for taxa other than *Phaeocystis*, however, might explain some of the more subtle results identified for uptake rates and stoichiometry. The less prominent members (e.g., diatoms and dinoflagellates) of the MMS community are taxa that become more dominant toward the end of the Ross Sea phytoplankton bloom, the time frame when the TNB experiment was conducted and when trends associated with changing the N:P supply ratio were identified. Community composition for the TNB experiment was not assessed; however, diatoms were likely not that abundant, because Si concentrations remained relatively constant in the treatments (Table 2). Results from both the TNB and MMS experiments indicate that late-season bloomers, particularly dinoflagellates, may be more sensitive to environmental stoichiometric changes.

## 4. Conclusions

Over the past few decades research has revealed a complex picture of elemental stoichiometry and how nutrient-microbe interactions structure the ratios that are observed in the water column as well as in phytoplankton. It is understood that there are feedback systems that work together to keep particulate and dissolved elemental ratios in a semistable state. The average C:N:P ratio of phytoplankton influences the dissolved ratios of the deep ocean, which can then be modified by microbial pathways (i.e., annamox and denitrification), which can lead to community shifts when the nutrients return to the surface. The feedbacks are linked to biogeochemical cycles and oceanic circulation, with the Southern Ocean exerting a regulatory force on oceanic nutrient inventories as deep water is formed and circulated globally. Shifts within the Southern Ocean phytoplankton community and the resulting impact on nutrient uptake rates and assimilation thus could have global implications for nutrient inventories over long time scales. Despite the Southern Ocean's critical role in global nutrient budgets, a comprehensive view of the factors that control and modify nutrient ratios of the Southern Ocean are lacking, let alone how stoichiometric changes in conjunction with other global change parameters may interact to influence ocean biogeochemistry. To our knowledge this is the first study from the Ross Sea to manipulate dissolved N:P ratios in combination with temperature and Fe and measure nutrient uptake rates. Our study represents a valuable stepping stone in understanding how dissolved N:P ratios individually and in combination with global change variables impact C and N cycles in the Ross Sea. In the two studies that we conducted, we present evidence that different dissolved N:P ratios have the potential to impact growth, nutrient utilization, and particulate ratios by microorganisms,

especially over short time scales. These trends were distinctly identified for the late season microbial community at Terra Nova Bay, but we also found that different dissolved N:P ratios resulted in subtle shifts to the less prominent members of the *Phaeocystis* dominated community from McMurdo Sound. By the end of both experiments, however, particulate ratios were generally similar among the treatments, and differences in nutrient utilization could be attributed to iron addition and/or temperature elevation. This demonstrates that homeostatic feedbacks within Southern Ocean phytoplankton communities minimize the impacts that changing dissolved N:P ratios has on particulate ratios, thus helping to maintain elemental ratios in a semistable state. It also indicates that in nutrient replete environments dissolved and particulate N:P ratios can become uncoupled and are not necessarily reflective of one another. Although dissolved stoichiometry may be an influential factor in shaping biogeochemical cycles and the composition of the microbial community, we found that other global change variables (temperature and Fe) were more significant drivers of change.

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## References

Anderson, T. R. (1994). Relating C:N ratios in zooplankton food and faecal pellets using a biochemical model. *Journal of Experimental Marine Biology and Ecology*, *184*, 183–199. https://doi.org/10.1016/0022-0981(94)90004-3

Arrigo, K. R. (2005). Marine microorganisms and global nutrient cycles. *Nature*, *437*, 349–355. https://doi.org/10.1038/nature04159

Arrigo, K. R., DiTullio, G. R., Dunbar, R. B., Robinson, D. H., VanWoert, M., Worthen, D. L., & Lizotte, M. P. (1999). Phytoplankton community structure and the drawdown of nutrients and $CO_2$ in the Southern Ocean. *Science*, *283*, 365–367. https://doi.org/10.1126/science.283.5400.365

Arrigo, K. R., DiTullio, G. R., Dunbar, R. B., Robinson, D. H., VanWoert, M., Worthen, D. L., & Lizotte, M. P. (2000). Phytoplankton taxonomic variability in nutrient utilization and primary production in the Ross Sea. *Journal of Geophysical Research*, *105*(C4), 8827–8846. https://doi.org/10.1029/1998JC000289

Aspila, K. I., & Agemian, C. (1976). A semi-automated method for determination of inorganic, organic and total phosphate in sediments. *Analyst*, *101*(1200), 187–197. https://doi.org/10.1039/an9760100187

Benitez-Nelson, C. R. (2000). The biogeochemical cycling of phosphorus in marine systems. *Earth Science Reviews*, *51*(1), 109–135. https://doi.org/10.1016/S0012-8252(00)00018-0

Berman-Frank, I., Cullen, J. T., Shaked, Y., Sherrell, R. M., & Falkowski, P. G. (2001). Iron availability, cellular iron quotas, and nitrogen fixation in *Trichodesmium*. *Limnology and Oceanography*, *46*, 1249–1260. https://doi.org/10.4319/lo.2001.46.6.1249

Bertrand, E. M., McCrow, J. P., Moustafa, A., Zheng, H., McQuaid, J. B., Delmont, T., et al. (2015). Phytoplankton-bacterial interactions mediate micronutrient colimination at the coastal Antarctic sea ice edge. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(32), 9938–9943. https://doi.org/10.1073/pnas.1501615112

Boyd, P. W., Dillingham, P. W., McGraw, C. M., Armstrong, E. A., Cornwall, C. E., Feng, Y.-y., et al. (2016). Physiological responses of a Southern Ocean diatom to complex future ocean conditions. *Nature Climate Change*, *6*, 207–213. https://doi.org/10.1038/nclimate2811

Boyd, P. W., Watson, A. J., Laws, C. S., Abraham, E. R., Trull, T., Murdoch, R., et al. (2000). A mesoscale phytoplankton bloom in the polar Southern Ocean stimulated by iron fertilization. *Nature*, *407*, 695–702. https://doi.org/10.1038/35037500

Bronk, D. A., Killbeg-Thoreson, L., Sipler, R. E., Mulholland, M. R., Roberts, Q. N., Bernhardt, P. W., et al. (2014). Nitrogen uptake and regeneration (ammonium regeneration, nitrification and photoproduction) in waters of the West Florida Shelf prone to blooms of *Karenia brevis*. *Harmful Algae*, *38*, 50–62. https://doi.org/10.1016/j.hal.2014.04.007

Cloern, J. E. (2001). Our evolving conceptual model of the coastal eutrophication problem. *Marine Ecology Progress Series*, *210*, 223–253. https://doi.org/10.3354/meps210223

Coale, K. H., Johnson, K. S., Chavez, F. P., Buesseler, K. O., Barber, R. T., Brezinski, M. A., et al. (2004). Southern Ocean iron enrichment experiment: carbon cycling in high- and low-Si waters. *Science*, *304*, 408–414. https://doi.org/10.1126/science.1089778

Cochlan, W. P. (2008). Nitrogen uptake in the Southern Ocean. In D. G. Capone, et al. (Eds.), *Nitrogen in the marine environment* (2nd ed., pp. 569–596). Oxford, UK: Academic Press, Elsevier.

Coello-Camba, A., & Agusti, S. (2017). Thermal thresholds of phytoplankton growth in polar waters and their consequences for a warming polar ocean. *Frontiers in Marine Science*, *4*, 168. https://doi.org/10.3389/fmars.2017.00168

Czerny, J. M. S., Hauss, H., Löscher, C., & Riebesell, U. (2016). Dissolved N:P ratio changes in the eastern tropical North Atlantic: effect on phytoplankton growth and community structure. *Marine Ecology Progress Series*, *545*, 49–62. https://doi.org/10.3354/meps11600

De Baar, H. J. W., Buma, A. G. J., Nolting, R. F., Cadée, G. C., Jacques, G., & Tréguer, P. J. (1990). On iron limitation of the Southern Ocean: experimental observations in the Weddell and Scotia Seas. *Marine Ecology Progress Series*, *65*, 105–122. https://doi.org/10.3354/meps065105

De Baar, H. J. W., Van Leeuwe, M. A., Scharek, R., Goeyens, L., Bakker, K. M. J., & Fritsche, P. (1997). Nutrient anomolies in Fragilariopsis kerguelensis blooms, iron deficiency and the nitrate/phosphate ratio (A. C. Redfield) of the Antarctic Ocean. *Deep Sea Research Part II*, *44*, 229–260. https://doi.org/10.1016/S0967-0645(96)00102-6

De Senerpont Domis, L. N., Van de Waal, D. B., Helmsing, N. R., Van Donk, E., & Mooij, W. M. (2014). Community stoichiometry in a changing world: combined effects of warming and eutrophication on phytopankton dynamics. *Ecology*, *95*(6), 1485–1495. https://doi.org/10.1890/13-1251.1

De La Rocha, C. L., Hutchins, D. A., Brezinski, M. A., & Zhang, Y. (2000). Effects of iron and zinc deficiency on elemental composition and silica production by diatoms. *Marine Ecology Progress Series*, *195*, 71–79. https://doi.org/10013/epic.22423

Delille, D. (2004). Abundance and function of bacteria in the Southern Ocean. *Cellular and Molecular Biology*, *50*(5), 543–551. https://doi.org/10.1170/T544

Deutsch, C., & Weber, T. (2012). Nutrient ratios as a tracer and driver of ocean biogeochemistry. *Annual Review of Marine Science*, *4*, 113–114. https://doi.org/10.1146/annurev-marine-120709-142821

DeVries, T., & Deutsch, C. (2014). Large-scale variations in the stoichiometry of marine organic matter respiration. *Nature Geoscience*, *7*, 890–894. https://doi.org/10.1038/NGEO2300

Dong, Z., Wang, X., & Liu, L. (2000). Wind erosion in arid and semiarid China: An overview. *Journal of Soil and Water Conservation*, *55*, 439–444.

Doucette, G. J., & Harrison, P. J. (1991). Aspects of iron and nitrogen nutrition in the red tide dinoflagellate *Gymnodinium sanguineum* I. Effects of iron depletion and nitrogen source on biochemical composition. *Marine Biology*, *110*(2), 165–173. https://doi.org/10.1007/BF01313701

Dugdale, R. C., & Wilkerson, F. P. (1986). The use of $^{15}N$ to measure nitrogen uptake in eutrophic oceans; experimental considerations. *Limnology and Oceanography*, *31*(4), 673–689. https://doi.org/10.4319/lo.1986.31.4.0673

Elser, J. J., Dobberfuhl, D. R., MacKay, N. A., & Schampel, J. H. (1996). Organism size, life history, and N:P stoichiometry. *BioScience*, *46*, 674–684. https://doi.org/10.2307/1312897

Feng, Y., Hare, C. E., Leblanc, K., Rose, J., Zhang, Y., DiTullio, G. R., et al. (2009). The effects of increased $pCO_2$ and temperature on the North Atlantic Spring Bloom: I. The phytoplankton community and biogeochemical response. *Marine Ecology Progress Series*, *388*, 13–25. https://doi.org/10.3354/meps08133

Feng, Y., Hare, C. E., Rose, J. M., Handy, S. M., DiTullio, G. R., Lee, P. A., et al. (2010). Interactive effects of iron, irradiance and $CO_2$ on Ross Sea phytoplankton. *Deep Sea Research Part I*, *57*(3), 368–383. https://doi.org/10.1016/j.dsr.2009.10.013

Finkel, Z. V., Beardall, J., Flynn, K. J., Quigg, A., Rees, T. A. V., & Raven, J. A. (2010). Phytoplankton in a changing world: cell size and elemental stoichiometry. *Journal of Plankton Research*, *32*(1), 119–137. https://doi.org/10.1093/plankt/fbp098

Fleming, R. H. (1940). The composition of plankton and units for reporting population and production. *Procedings of Sixth Pacific Science Congress California*, *3*, 535–540.

Geider, R. J., & La Roche, J. (2002). Redfield revisited: variability of C:N:P in marine microalgae and its biochemical basis. *European Journal of Phycology*, *37*(1), 1–17. https://doi.org/10.1017/S0967026201003456

Gledhill, M., & Buck, K. N. (2012). The organic complexation of iron in the marine environment: A review. *Frontiers in Microbiology*, *3*, 69. https://doi.org/10.3389/fmicb.2012.00069

Goldman, J. C., McCarthy, J. J., & Peavey, D. G. (1979). Growth-rate influence on the chemical composition of phytoplankton in oceanic waters. *Nature*, *279*, 210–215. https://doi.org/10.1038/279210a0

Gruber, N., & Deutsch, C. A. (2014). Redfield's evolving legacy. *Nature Geoscience*, *7*, 853–855. https://doi.org/10.1038/ngeo2308

Hama, T., Miyazaki, T., Ogawa, Y., Iwakuma, T., Takahashi, M., Otsuki, A., & Ichimura, S. (1983). Measurement of photosynthetic production of a marine phytoplankton population using a stable $^{13}C$ isotope. *Marine Biology*, *73*, 31–36. https://doi.org/10.1007/BF00396282

Hansell, D. A. (2005). Dissolved organic carbon reference material program. *Eos, Transactions American Geophysical Union*, *86*, 318–318. https://doi.org/10.1029/2005EO350003

Hare, C. E., Leblanc, K., DiTullio, G. R., Kudela, R. M., Zhang, Y., Lee, P. A., et al. (2007). Consequences of increased temperature and $CO_2$ for algal community structure and biogeochemistry in the Bering Sea. *Marine Ecology Progress Series*, *352*, 9–16. https://doi.org/10.3354/meps07182

Heckey, R. E., Campbell, P., & Hendzel, L. L. (1993). The stoichiometry of carbon, nitrogen, and phosphorus in particulate matter of lakes and oceans. *Limnology and Oceanography*, *38*, 709–724. https://doi.org/10.4319/lo.1993.38.4.0709

Hutchins, D. A., & Boyd, P. W. (2016). Marine phytoplankton and the changing ocean iron cycle. *Nature Climate Change*, *6*, 1071–1079. https://doi.org/10.1038/nclimate3147

Hutchins, D. A., & Fu, F. X. (2017). Microorganisms and ocean global change. *Nature Microbiology*, *2*, 17508. https://doi.org/10.1038/nmicrobiol.2017.58

Hutchins, D. A., Witter, A. E., Butler, A., & Luther, G. W. III (1999). Competition among marine phytoplankton for different chelated iron species. *Nature*, *400*, 858–861. https://doi.org/10.1038/23680

Intergovernmental Panel on Climate Change (2014). In Core Writing Team. In R. K. Pachauri & L. A. Meyer (Eds.), *Climate change 2014: Synthesis report. contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Geneva, Switzerland: IPCC.

Jørgensen, N. O. G., Kroer, N., Coffin, R. B., Yang, X.-H., & Lee, C. (1993). Dissolved free amino-acids, combined amino-acids, and DNA as sources of carbon and nitrogen to marine bacteria. *Marine Ecology Progress Series*, *98*, 135–148.

Karl, D. M., Letelier, R., Hebel, D., Tupas, L., Dore, J., Christian, J., & Winn, C. (1995). Ecosystem changes in the North Pacific subtropical gyre attributed to the 1991–92 El Niño. *Nature*, *373*, 230–234. https://doi.org/10.1038/373230a0

Keil, R. G., & Kirchman, D. L. (1999). Utilization of dissolved protein and amino acids in the northern Sargasso Sea. *Aquatic Microbial Ecology*, *18*, 293–300. https://doi.org/10.3354/ame018293

King, A. L., Sanudo-Wilhelmy, S. A., Leblanc, K., Hutchins, D. A., & Fu, F.-X. (2011). $CO_2$ and vitamin $B_{12}$ interactions determine bioactive trace metal requirements of a subarctic Pacific diatom. *The ISME Journal*, *5*, 1388–1396. https://doi.org/10.1038/ismej.2010.211

Klausmeier, C. A., Litchman, E., Daufresne, T., & Levin, S. A. (2004). Optimal nitrogen-to-phosphorus stoichiometry of phytoplankton. *Nature*, *429*, 171–174. https://doi.org/10.1038/nature02454

Klein Breteler, W. C. M., Schogt, N., & Rampen, S. (2005). Effect of diatom nutrient limitation on copepod development: role of essential lipids. *Marine Ecology Progress Series*, *291*, 125–133. https://doi.org/10.3354/meps291125

Koroleff, F. (1983). Determination of nutrients. In K. Grasshoff, et al. (Eds.), *Methods of seawater analysis* (pp. 125–187). New York: Verlag Chemie.

Lagus, A., Suomela, J., Weithoff, G., Heikkilä, K., Helminen, H., & Sipura, J. (2004). Species-specific differences in phytoplankton responses to N and P enrichments and the N:P ratio in the Archipelago Sea, northern Baltic Sea. *Journal of Plankton Research*, *26*(7), 779–798. https://doi.org/10.1093/plankt/fbh070

Lomas, M. W., & Glibert, P. M. (2000). Comparisons of nitrate uptake, storage, and reduction in marine diatoms and flagellates. *Journal of Phycology*, *36*(5), 903–913. https://doi.org/10.1046/j.1529-8817.2000.99029.x

Maldonado, M. T., & Price, N. M. (1996). Influence of N substrate on Fe requirements of marine centric diatoms. *Marine Ecology Progress Series*, *141*, 161–172. https://doi.org/10.3354/meps141161

Martin, J. H., Fitzwater, S. E., & Gordon, R. M. (1990). Iron definciency limits phytoplankton growth in Antarctic waters. *Global Biogeochemical Cycles*, *4*, 5–12. https://doi.org/10.1038/331341a0

Martiny, A. C., Pham, C. T. A., Primeau, F. W., Vrugt, J. A., Moore, J. K., Levin, S. A., & Lomas, M. W. (2013). Strong latitudinal patterns in the elemental ratios of marine plankton and organic matter. *Nature Geoscience*, *6*, 279–283. https://doi.org/10.1038/ngeo1757

McMinn, A., Ashworth, C., & Ryan, K. G. (2000). In Situ net primary productivity of an Antarctic fast ice bottom algal community. *Aquatic Microbial Ecology*, *21*, 177–185. https://doi.org/10.3354/ame021177

Moore, C. M., Mills, M. M., Arrigo, K. R., Berman-Frank, I., Bopp, L., Boyd, P. W., et al. (2013). Processes and patterns of oceanic nutrient limitation. *Nature Geoscience*, *6*(9), 701–710. https://doi.org/10.1038/ngeo1765

Mulholland, M. R., & Lomas, M. W. (2008). Nitrogen uptake and assimilation. In D. G. Capone, et al. (Eds.), *Nitrogen in the marine environment* (2nd ed., pp. 303–361). Oxford, UK: Academic Press, Elsevier.

Pahlow, M., & Riebesell, U. (2000). Temporal trends in deep ocean Redfield ratios. *Science*, *287*, 831–833. https://doi.org/10.1126/science.287.5454.831

Paing, J., Gomez, E., & Picot, B. (1999). Humic substances interactions with sedimentary phosphorus. *Analusis*, *27*(5), 436–438. https://doi.org/10.1051/analusis:1999270436

Parsons, T., Maita, Y., & Lalli, C. (1984). *A manual of chemical and biological methods for seawater analysis*. New York: Pergamon Press.
Paytan, A., & McLaughlin, K. (2007). The oceanic phosphorus cycle. *Chemical Reviews*, *107*, 563–576. https://doi.org/10.1021/cr0503613
Petrou, K., Kranz, S. A., Trimborn, S., Hassler, C. S., Ameijeiras, S. B., Sackett, O., et al. (2016). Southern Ocean phytoplankton physiology in a changing climate. *Journal of Plant Physiology*, *203*, 135–150. https://doi.org/10.1016/j.jplph.2016.05.004
Planavsky, N. J. (2014). The elements of marine life. *Nature Geoscience*, *7*, 855–856. https://doi.org/10.1038/ngeo2307
Pomeroy, L. W., & Wiebe, W. J. (2001). Temperature and substrates as interactive limiting factors for marine heterotrophic bacteria. *Aquatic Microbial Ecology*, *23*, 187–204. https://doi.org/10.3354/ame023187
Price, N., & Harrison, P. (1987). A comparison of methods for the measurement of dissolved urea concentrations in seawater. *Marine Biology*, *94*, 307–317. https://doi.org/10.1007/BF00392945
Quigg, A., Finkel, Z. V., Irwin, A. J., Rosenthal, Y., Ho, T.-Y., Reinfelder, J. R., et al. (2003). The evolutionary inheritance of elemental stoichiometry in marine phytoplankton. *Nature*, *425*, 291–294. https://doi.org/10.1098/rspb.2010.1356
Quigg, A., Irwin, A. J., & Finkel, Z. V. (2011). Evolutionary inheritance of elemental stoichiometry in phytoplankton. *Proceedings of the Royal Society of London. Series B*, *278*(1705), 526–534. https://doi.org/10.1098/rspb.2010.1356
Raiswell, R., Benning, L. G., Tranter, M., & Tulaczyk, S. (2008). Bioavailable iron in the Southern Ocean: The significance of the iceberg conveyor belt. *Geochemical Transactions*, *9*, 7. https://doi.org/10.1186/1467-4866-9-7
Reay, D. S., Priddle, J., Nedwell, D. B., Whitehouse, M. J., Ellis-Evans, J. C., Deubert, C., & Connelly, D. P. (2001). Regulation by low temperature of phytoplankton growth and nutrient uptake in the Southern Ocean. *Marine Ecology Progress Series*, *219*, 51–64. https://doi.org/10.3354/meps219051
Redfield, A. C. (1934). On the proportions of organic derivatives in seawater and their relation to the composition of plankton. In R. J. Daniel (Ed.), *James Johnstone Memorial Volume* (pp. 176–192). Liverpool: University Press.
Rhee, G. Y. (1978). Effects of N:P atomic ratios and nitrate limitation on algal growth, cell composition, and nitrate uptake. *Limnology and Oceanography*, *23*(1), 10–25. https://doi.org/10.4319/lo.1978.23.1.0010
Rivkin, R. B., & Putt, M. (1987). Heterotrophy and photoheterotrophy by Antarctic microalgae: light dependent incorporation of amino acids and glucose. *Journal of Phycology*, *23*(3), 442–452. https://doi.org/10.1111/j.1529-8817.1987.tb02530.x
Rose, J. M., Feng, Y., DiTullio, G. R., Dunbar, R. B., Hare, C. E., Lee, P. A., et al. (2009). Synergistic effects of iron and temperature on Antarctic phytoplankton and microzooplankton assemblages. *Biogeosciences*, *6*, 3131–3147. https://doi.org/10.5194/bg-6-3131-2009
Rousseau, V., Mathot, S., & Lancelot, C. (1990). Calculating carbon biomass of *Phaeocystis* sp. from microscopic observations. *Marine Biology*, *107*, 305. https://doi.org/10.1007/BF01319830
RStudio Team (2015). RStudio: Integrated Development for R. RStudio, Inc., Boston, MA. Retrieved from http://www.rstudio.com/
Schoemann, V., Becquebvort, S., Stefels, J., Rousseau, V., & Lancelot, C. (2005). *Phaeocystis* blooms in the global ocean and their controlling mechanisms: A review. *Journal of Sea Research*, *53*, 43–66. https://doi.org/10.1016/j.seares.2004.01.008
Scott, F. J., & Marchant, H. J. (Eds.) (2005). Antarctic marine protists. In *Australian biological resources study* (pp. 13–201). Hobart: Canberra and Australian Antarctic Division.
Sedwick, P. N., & DiTullio, G. R. (1997). Regulation of algal blooms in Antarctic shelf waters by the release of iron from melting sea ice. *Geophysical Research Letters*, *24*(20), 2515–2518. https://doi.org/10.1029/97GL02596
Sedwick, P. N., Marsay, C. M., Sohst, B. M., Aguilar-Islas, A. M., Lohan, M. C., Long, M. C., et al. (2011). Early season depletion of dissolved iron in the Ross Sea polynya: Implications for iron dynamics on the Antarctic continental shelf. *Journal of Geophysical Research*, *116*, C12019. https://doi.org/10.1029/2010JC006553
Sharp, J. H., Beauregard, A. Y., Burdige, D., Cauwet, G., Curless, S. E., Lauck, R., et al. (2004). A direct instrument comparison for measurement of total dissolved nitrogen in seawater. *Marine Chemistry*, *84*, 181–193. https://doi.org/10.1016/j.marchem.2003.07.003
Simon, M., & Rosenstock, B. (2007). Different coupling of dissolved amino acid, protein, and carbohydrate turnover to heterotrophic picoplankton production in the Southern Ocean in Austral Summer and Fall. *Limnology and Oceanography*, *52*(1), 85–95. https://doi.org/10.4319/lo.2007.52.1.0085
Smith, W. O., Marra, J., Hiscock, M. R., & Barber, R. T. (2000). The seasonal cycle of phytoplankton biomass and primary productivity in the Ross Sea, Antarctica. *Deep Sea Research Part II*, *47*, 3119–3140. https://doi.org/10.1016/S0967-0645(00)00061-8
Solomon, C. M., Lessard, E. J., Keil, R. G., & Foy, M. S. (2003). Characterization of extracellular polymers of *Phaeocystis globosa* and *P. antarctica*. *Marine Ecology Progress Series*, *250*, 81–89. https://doi.org/10.3354/meps250081
Spackeen, J. L., Sipler, R. E., Bertrand, E. M., Xu, K., McQuaid, J. B., Walworth, N. G., et al. (2018). Impact of temperature, $CO_2$, and iron on nutrient uptake by a late-season microbial community from the Ross Sea, Antarctica. *Aquatic Microbial Ecology*. https://doi.org/10.3354/ame01886
Sterner, R. W., & Elser, J. J. (2002). *Ecological stoichiometry: The biology of elements from molecules to the biosphere*. Princeton, NJ: Princeton University Press.
Sunda, W. G., Price, N. M., & Morel, F. M. M. (2005). Trace metal ion buffers and their use in culture studies. In R. A. Andersen (Ed.), *Algal culturing techniques* (pp. 35–63). Burlington: Elsevier Academic Press.
Suttle, C. A., Chan, A. M., & Furham, J. A. (1991). Dissolved free amino-acids in the Sargass Sea: uptake and resiration rates, turnover times, and concentrations. *Marine Ecology Progress Series*, *70*(2), 189–199.
Tatters, A. O., Roleda, M. R., Schnetzer, A., Fu, F.-X., Hurd, C. L., Boyd, P. W., et al. (2013). Short- and long-term conditioning of a temperate marine diatom community to acidification and warming. *Philosophical Transactions of the Royal Society of London: Series B*, *368*, 20120437. https://doi.org/10.1098/rstb.2012.0437
Teng, Y.-C., Primeau, F. W., Moore, J. K., Lomas, M. W., & Martiny, A. C. (2014). Global-scale variations of the ratios of carbon to phosphorus in exported marine organic matter. *Nature Geoscience*, *7*, 895–898. https://doi.org/10.1038/ngeo2303
Tilman, D., Kiesling, R. L., Sterner, R., Kilham, S. S., & Johnson, F. A. (1986). Green, bluegreen and diatom algae: taxonomic differences in competitive ability for phosphorus, silicon, and nitrogen. *Archiv für Hydrobiologie*, *106*, 473–485.
Tomas, C. R. (1997). *Identifying marine phytoplankton*. New York: Academic Press.
Tyrell, T. (1999). The relative influences of nitrogen and phosphorus on oceanic primary production. *Nature*, *400*, 525–538. https://doi.org/10.1038/22941
Utermöhl, H. (1931). Neue Wege in der quantitativen Erfassung des Plank- tons, (Mit besonderer Beriicksichtigung des Ultraplanktons). *Verhandlungen des Internationalen Verein Limnologie*, *5*, 567–596.
Wang, X., Tang, K. W., Wang, Y., & Smith, W. O. Jr. (2010). Temperature effects on growth, colony development and carbon partitioning in three *Phaeocystis* species. *Aquatic Biology*, *9*, 239–249. https://doi.org/10.3354/ab00256
Welshmeyer, N. (1994). Fluorometric analysis of chlorophyll a in the presence of chlorophyll b and phaeopigments. *Limnology and Oceanography*, *39*(8), 1985–1992. https://doi.org/10.4319/lo.1994.39.8.1985

Xu, K., Fu, F.-X., & Hutchins, D. A. (2014). Comparative responses of two dominant Antarctic phytoplankton taxa to interactions between ocean acidification, warming, irradiance, and iron availability. *Limnology and Oceanography*, *59*(6), 1919–1931. https://doi.org/10.4319/lo.2014.59.6.1919

Zhu, Z., Xu, K., Fu, F.-X., Spackeen, J. L., Bronk, D. A., & Hutchins, D. A. (2016). A comparative study of iron and temperature interactive effects on diatoms and *Phaeocystis antarctica* from the Ross Sea, Antarctica. *Marine Ecology Progress Series*, *550*, 39–51. https://doi.org/10.3354/meps11732

Zubkov, M. V., Tarran, G. A., Mary, I., & Fuchs, B. M. (2008). Differential microbial uptake of dissolved amino acids and amino sugars in surface waters of the Atlantic Ocean. *Journal of Plankton Research*, *30*(2), 211–220. https://doi.org/10.1093/plankt/fbm091