Impact of Climate Change Variables on Nutrient Cycling by Marine Microorganisms in the Southern California Bight and Ross Sea, Antarctica

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IMPACT OF CLIMATE CHANGE VARIABLES ON
NUTRIENT CYCLING BY MARINE MICROORGANISMS IN THE SOUTHERN
CALIFORNIA BIGHT AND ROSS SEA, ANTARCTICA

A Dissertation
Presented to

The Faculty of the School of Marine Science
The College of William and Mary in Virginia

In Partial Fulfillment
of the Requirements for the Degree of
Doctor of Philosophy

by
Jenna Lee Spackeen
August 2017
APPROVAL SHEET

This dissertation is submitted in partial fulfillment of
The requirements for the degree of

Doctor of Philosophy

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DEDICATION

To my Momma and Bandida for providing me with unconditional love and support. Throughout my graduate school journey you have both been my rock. Thanks for coming with me on a ride that has definitely proven to be one “long, strange trip.”
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AUTHOR’S NOTE

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DISSERTATION ABSTRACT

Ocean environments are being impacted by climate warming, elevated carbon dioxide (CO$_2$) levels, and shifting nutrient sources and sinks. It is essential to quantify the sensitivity of microorganisms to these effects of global change because they form the base of the marine food web and are an integral component of nutrient cycling on the planet. Their role in photosynthesis, nutrient uptake, and transfer of organic matter into higher trophic levels or to the deep ocean via the biological pump render microorganisms key in ecosystem structure and function and in regulating the global climate. The goal of this dissertation research was to determine how changing environmental conditions impact microbial communities and the rates at which they take up nutrients. Research for this dissertation took place in the Southern California Bight and in the Ross Sea, Antarctica, where fully factorial designs were used to investigate the response of microorganisms to multiple global change parameters. Nutrient uptake rates were measured using $^{13}$C and $^{15}$N stable isotopes for carbon and nitrogen substrates and $^{33}$P radioisotopes for phosphorus substrates.

In the Southern California Bight, a microbial assemblage was collected and incubated in an ‘ecostat’ continuous culture system, where elevated temperature, CO$_2$, and the dominant nitrogen substrate (nitrate or urea) in the diluent were manipulated. During this experiment uptake rates of dissolved inorganic carbon (DIC), nitrate (NO$_3^-$), and urea were determined for two microbial size classes (0.7-5.0 µm and >5.0 µm). Urea uptake rates were greater than NO$_3^-$, and uptake rates of urea and DIC for both size fractions increased at elevated temperature, while uptake rates of NO$_3^-$ by smaller microorganisms increased when CO$_2$ levels were high.

In the Ross Sea, the impact of elevated temperature, CO$_2$, and iron addition on DIC and NO$_3^-$ uptake rates by two size classes (0.7-5.0 µm and >5.0 µm) of a late-season microbial community were investigated using a semi-continuous and continuous ‘ecostat’ culturing approach. Temperature impacted the microbial community the most, significantly increasing NO$_3^-$ and DIC uptake rates by larger microorganisms. The effects of iron addition were more apparent when temperature was also elevated, and CO$_2$ did not impact rates. Bioassay experiments were also conducted in the Ross Sea to determine how increasing and decreasing the N:P supply ratio in combination with other parameters (temperature and iron) impact uptake rates of DIC, NO$_3^-$, and amino acids. Results from these experiments show that changes to the dissolved N:P supply ratio have the potential to alter nutrient uptake rates over short time scales, but that temperature elevation and iron addition have a larger impact. Additional experiments were completed on diatoms (*Fragilariopsis cylindrus* and *Pseudo-nitzschia subcurvata*) and *Phaeocystis antarctica*, three important phytoplankton species collected from the Ross Sea, to assess how temperature elevation and iron addition impact uptake rates of a number of inorganic and organic carbon, nitrogen, and phosphorus substrates. These culture studies generally show that when temperature is increased, diatoms are able to take up nutrients more rapidly than *Phaeocystis antarctica*. 
Results from this dissertation show that nutrient cycles and phytoplankton communities in the Southern California Bight and the Ross Sea, Antarctica will likely be different in the future. Although all variables tested were found to exert some influence on microbial nutrient cycling, temperature elevation generally had the largest effect, increasing biomass and uptake rates, structuring the composition of the microbial community, and altering stoichiometry. This research did not include top down effects and it is limited spatially and temporally, however, it demonstrates the importance of studying different nutrient substrates and looking at multiple interactive stressors to gain a more comprehensive view of potential change.
IMPACT OF CLIMATE CHANGE VARIABLES ON
NUTRIENT CYCLING BY MARINE MICROORGANISMS IN THE SOUTHERN
CALIFORNIA BIGHT AND ROSS SEA, ANTARCTICA
CHAPTER 1

INTRODUCTION
Importance of Phytoplankton and their role in Global Nutrient Cycles

Marine microalgae, or phytoplankton, play a critical role in biogeochemical cycles on our planet (Arrigo 2005). Accounting for approximately half of global primary production (Field et al. 1998), phytoplankton directly contribute to the carbon cycle as carbon dioxide (CO$_2$) is consumed and oxygen is released during photosynthesis. In addition to taking up CO$_2$, phytoplankton also need other nutrients for growth, including nitrogen, phosphorus, and micronutrients such as iron (Fe). For certain phytoplankton groups (i.e., diatoms), silica is an additional requirement. Phytoplankton form the base of the marine food web and are responsible for the transfer of nutrients into higher trophic levels. They also transport carbon, and other nutrients, to the deep ocean via the biological pump (Arrigo 2005).

Marine phytoplankton communities are comprised of an interacting group of phytoplankton species in a common location. Common phytoplankton groups include diatoms, dinoflagellates, haptophytes, chlorophytes, coccolithophores, and cyanobacteria. Phytoplankton groups employ different strategies for growth and survival and have varying nutrient requirements. These variations are reflected in the rates at which they take up and utilize nutrient substrates and in their elemental cellular composition. The majority of phytoplankton are able to take up a variety of nutrient substrates, however, some groups are able to utilize certain substrates more effectively than others. For example, ammonium (NH$_4^+$), a reduced form of nitrogen, is often assumed to be the ideal nitrogen substrate because it requires less energy to incorporate than nitrate (NO$_3^-$) and nitrite (NO$_2^-$), which are oxidized forms of nitrogen (McCarthy 1981; Glibert et al. 2016 and references therein). Diatoms, however, have evolved strategies to effectively take up...
NO$_3^-$ and are known to have a high affinity for NO$_3^-$ at low concentrations (Cochlan et al. 2008). Furthermore, it was historically thought that organic pools of nutrients were not readily used by phytoplankton. Research over the last few decades has revealed that turnover rates of dissolved organic forms of carbon, nitrogen, and phosphorus can be high, and in some cases uptake rates of organic nutrient forms can exceed uptake rates of inorganic nutrients (e.g. Fu et al. 2005; Bronk et al. 2007; Paytan & McLaughlin 2007; Solomon et al. 2010; Sipler & Bronk 2015; Michelou et al. 2011).

In this dissertation, phytoplankton groups of interest are diatoms and haptophytes, specifically *Phaeocystis antarctica*. Diatoms (division Bacillariophyta) are considered one of the most important phytoplankton groups, because they are responsible for one quarter of global primary production (Field et al. 1998; Boyd et al. 2012). Diatoms consist of two distinct morphological types—pennate, which are bilaterally symmetrical and tend to be smaller, and centric, which have radial symmetry and tend to be larger. Diatoms are commonly found in coastal locations, where concentrations of nutrients are relatively high and NO$_3^-$ is present. Diatoms use silica to build their frustules and are, thus, important in regulating the planetary silica cycle. *Phaeocystis antarctica* strongly influences biogeochemical cycles and is a major source of dimethylsulfide, a gas that is climatically important because it is involved in cloud formation (Gibson et al. 1990).

**Global Change Parameters**

Our planet is currently experiencing changes due to natural causes and anthropogenic perturbations (Hoegh-Guldberg & Bruno 2010) that have the potential to impact phytoplankton physiology and global biogeochemical cycles. Temperature is one
of the main variables changing on our planet, and it is one of the primary parameters investigated in this dissertation. Increased emissions from combustion of fossil fuels, selective agricultural practices, and deforestation have and will continue to release greenhouse gases, including CO$_2$, into the atmosphere (Solomon et al. 2007; Doney & Schimel 2007). Higher atmospheric concentrations of greenhouse gases are increasing average surface temperatures at a rate that is unprecedented relative to Earth’s geologic past (Trenberth et al. 2007). Global surface temperatures are currently predicted to increase by 1.4 to 5.8°C over the next century and atmospheric concentrations of CO$_2$ are expected to reach approximately 800 ppm (IPCC 2014).

A temperature increase of a few degrees typically increases microbial growth rates and photosynthesis (Eppley 1972; Geider 1987; Raven & Geider 1988; Davison 1991; Lomas & Glibert 1999; Berges et al. 2002; Boyd et al. 2013), however, phytoplankton species vary in their optimum temperature for growth and in their thermal tolerance range (i.e., temperature range where growth rate is positive; Thomas et al. 2012). As sea surface temperatures rise, certain phytoplankton species and/or groups may be at a competitive advantage. Temperature is one of the strongest predictors of diatom primary productivity (Boyd et al. 2016), and diatoms can outcompete other groups when grown under simulated future conditions where temperature is increased (Rose et al. 2009; Xu et al. 2014).

Similar to temperature, some phytoplankton species and/or groups may be better adapted to live in a high CO$_2$ ocean (Riebesell 2004; Doney et al. 2009). Most phytoplankton species possess carbon concentrating mechanisms (CCMs), an apparatus used to concentrate dissolved inorganic carbon (DIC) in cells to allow high rates of
photosynthesis (Colman & Rotatore 1995; Reinfelder 2011). As the partial pressure of ambient CO$_2$ increases, the need for CCMs may decrease, which will allow the energy expended to operate the CCMs to be allocated toward other cellular functions (Raven 1991). Microorganisms that do not have CCMs and obtain DIC via passive diffusion may also benefit as ambient DIC concentrations increase (Beardall et al. 2009). Studies find that high CO$_2$ conditions can impact cellular physiology (Tortell et al. 2008, 2010; McMinn et al. 2014), thus, favoring certain species of phytoplankton over others (Feng et al. 2010; Hoppe et al. 2013). Other studies, however, find that CO$_2$ does not have a noticeable impact on cellular physiology (Boelen et al. 2011; Crawfurd et al. 2011; Young et al. 2015).

Uncertainties surrounding phytoplankton responses to both elevated temperature and high CO$_2$ conditions highlight the need for research in this area across different marine systems. This dissertation research was conducted in a temperate location, the Southern California Bight, and a polar region, the Ross Sea, Antarctica. At both study sites, the effects of temperature and CO$_2$ were examined in addition to nutrient sources relevant to each location.

**The Southern California Bight**

The Southern California Bight extends from Point Conception to San Diego, and includes the offshore Channel Islands. This coastal region is densely populated with humans – nearly seven million people reside in the cities of Los Angeles and San Diego. Nutrient concentrations in the Southern California Bight are influenced by upwelling, which brings nutrients up to the surface from deeper water, and terrestrial sources, such
as runoff or riverine discharge. Over the past few decades, nutrient sources in this region have been modified. Models show that the supply of NO$_3^-$ to coastal California waters has increased since 1980 (Jacox et al. 2015), and the supply of NO$_3^-$ is expected to continue to increase substantially over the next century due to fluxes from deep waters (Rykaczewski & Dunne 2010). With respect to terrestrial sources, excess delivery of nutrients from agricultural practices and wastewater is a concern. Harmful algal blooms have become a recurring issue off the coast of California in recent decades (Schnetzer et al. 2007), and one frequent bloomer, *Pseudo-nitzschia* spp., is capable of producing toxic domoic acid (Bates et al. 1989; Bates & Trainer 2006; Schroeder et al. 2015).

Agricultural runoff and wastewater both contain urea (Glibert et al. 2006), an organic form of nitrogen, so it is particularly important to consider how Southern California Bight microorganisms, including *Pseudo-nitzschia* spp., respond to urea.

**The Ross Sea**

The Southern Ocean comprises 20% of the global ocean making it a major ocean biome (Boyd 2002). The Southern Ocean as a whole is one of the most biologically productive locations on our planet, and the Ross Sea in particular is one of the most productive Antarctic regions, contributing as much as 28% to Southern Ocean total primary production (Arrigo et al. 1998). Seasonally high rates of primary production coupled with high CO$_2$ solubility, due to the cold surface waters, make this region an important global CO$_2$ sink. Research shows that the Southern Ocean accounts for approximately a quarter of the oceanic uptake of CO$_2$ (Arrigo et al. 2008). Although the region is highly productive, most of the Southern Ocean, including the Ross Sea, is
considered a high nutrient low chlorophyll (HNLC) region, and primary production is limited by Fe. However, a predicted consequence of climate change is increased supply of Fe to the Southern Ocean (Sedwick and DiTullio 2007). In the Ross Sea, meltwater from glaciers or icebergs will deliver bioavailable Fe to the sea (Raiswell et al. 2008). Another mechanism of Fe delivery to the Ross Sea is through eolian transport. More soil on the Antarctic continent will be exposed as snow cover retreats (Jacobs et al. 2002) and the delivery of eolian dust from other continents, such as Australia, may increase with warming (Revel-Rolland et al. 2006). The Southern Ocean is highly sensitive to change and environmental modifications are already occurring across the region, including atmospheric warming and rising sea surface temperatures (SST), higher concentrations of CO$_2$ in both the atmosphere and ocean, and significant loss of sea ice (IPCC 2014; Constable et al. 2014 and references therein). How phytoplankton will respond to such changes and how nutrient cycling may be altered will largely be dependent upon Fe and its availability in the future.

**Dissertation Structure and Objectives**

This dissertation aimed to determine how a variety of global change parameters, individually and synergistically, affect nutrient cycling by marine microorganisms in the Southern California Bight and the Ross Sea, Antarctica. Chapters 2 through 5 are the main chapters of the dissertation, and the objective and general approach for these chapters are described below.

*Chapter 2* examines how elevated temperature and increased CO$_2$ levels, individually and in combination, impact uptake rates of NO$_3^-$, urea, and DIC by different
size fractions (0.7 – 5.0 µm and > 5.0 µm) of a microbial community collected from the Southern California Bight. A natural microbial assemblage was collected and incubated for 10 days using an ‘ecostat’ continuous culture system that supplied the community with diluent that contained either NO₃⁻ or urea as the dominant nitrogen substrate. Throughout the experiment, biomass parameters, nutrient concentrations, and uptake rates were measured.

**Chapter 3** examines the individual and combined effects of elevated temperature, increased CO₂, and Fe addition on uptake rates of NO₃⁻ and DIC by different size fractions (0.7 – 5.0 µm and > 5.0 µm) of a late-season microbial community collected from the Ross Sea, Antarctica. Using both a semi-continuous and ‘ecostat’ continuous culturing approach, an intact microbial assemblage was incubated for nearly a month, and biomass parameters, nutrient concentrations, and uptake rates were measured.

**Chapter 4** examines how altered N:P supply ratios in the Ross Sea in combination with other global change parameters, including Fe addition and elevated temperature, impact microbial nutrient utilization and community composition. Two experiments were completed in the Ross Sea using microbial communities collected from Terra Nova Bay and McMurdo Sound. Microorganisms were cultured in batch mode, and uptake rates of NO₃⁻, DIC, and amino acids were determined for two different microbial size fractions (0.7 – 5.0 µm and > 5.0 µm). For the experiment that used McMurdo Sound microorganisms, differences in community composition between the treatments were also assessed.

**Chapter 5** examines how elevated temperature and Fe addition, individually and in combination, impact uptake rates of inorganic and organic carbon, nitrogen, and
phosphorus substrates by three different Ross Sea phytoplankton species – two pennate diatoms (*Fragilariopsis cylindrus* and *Pseudo-nitzschia subcurvata*) and a haptophyte (*Phaeocystis antarctica*). Phytoplankton species were isolated from the Ross Sea and cultured for an extended period of time under these conditions. Uptake rates and elemental stoichiometry of the species were assessed.

**Broader Implications**

Phytoplankton play a critical role in the cycling of nutrients in the marine environment. Phytoplankton primary productivity is a large sink for atmospheric CO₂, with the potential to be removed from surface waters through the biological pump. Despite the importance of phytoplankton, a comprehensive understanding of how global change affects phytoplankton communities and biogeochemical cycling is lacking. Modifications to the nutrient sources of an ecosystem have the potential to affect primary production and alter the global cycling of nutrients by causing shifts in community structure and subsequent modifications to nutrient pathways (Arrigo 2005). Uptake of nutrients by phytoplankton will likely be affected in the future due to several global change parameters, including elevated temperature, increased concentrations of CO₂, and modifications to an environment’s nutrient sources. Quantifying rates of nutrient use through uptake experiments under expected future conditions allows us to predict how nutrient cycles will change over the next few centuries, and the data gathered can be used in biogeochemical models to address global change questions.


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CHAPTER 2

INTERACTIVE EFFECTS OF ELEVATED TEMPERATURE AND CO₂ ON NITRATE, UREA, AND DIC UPTAKE BY A COASTAL CALIFORNIA MICROBIAL COMMUNITY

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ABSTRACT: Average global temperatures and carbon dioxide (CO₂) levels are expected to increase in the coming decades. Implications for ocean ecosystems include shifts in microbial community structure and subsequent modifications to nutrient pathways. Studying how predicted future temperature and CO₂ conditions will impact the biogeochemistry of the ocean is important because of the ocean’s role in regulating global climate. We determined how elevated temperature and CO₂ affect uptake rates of nitrate, urea, and dissolved inorganic carbon by two size classes (0.7-5.0 µm and >5.0 µm) of a microbial assemblage collected from coastal California. This microbial community was incubated for 10 days using an ecostat continuous culture system that supplied the microorganisms with either nitrate or urea as the dominant nitrogen source. Biomass parameters, nutrient concentrations, and uptake rates were measured throughout the experiment. In all treatments, urea uptake rates were greater than nitrate, and larger microorganisms had higher uptake rates than smaller microorganisms. Uptake rates of urea and dissolved inorganic carbon within both size fractions were higher at elevated temperature, and uptake rates of nitrate by smaller microorganisms increased with elevated CO₂. These findings suggest that the rate at which nutrients cycle in temperate coastal waters will increase as temperature and CO₂ levels rise, and that the affect will vary between nitrogen substrates and different microorganisms.
INTRODUCTION

Global temperatures and atmospheric concentrations of carbon dioxide (CO₂) are increasing at unprecedented rates compared to Earth’s geologic past (Beardall et al. 2009) with the rise predicted to continue in the future (Cubasch & Wuebbles 2014). Depending on the model, average global surface temperature is currently predicted to increase by 1.4 to 5.8°C over the next century (IPCC 2014), and atmospheric CO₂ levels are projected to reach 750 to 1000 ppm by 2100 (Meehl et al. 2007). Considering the magnitude of the predicted change, it is crucial to understand how marine microorganisms will respond to increasing temperature and CO₂ at both physiological and ecological levels, because they form the base of the marine food web and largely control the cycling of nutrients throughout the ocean (Boyd & Hutchins 2012).

Temperature changes will affect the physiology of microorganisms, as well as their global distribution. For example, microorganisms capable of fixing nitrogen typically live in warmer oceans, and their distribution may expand as the temperature of the surface water increases (Boyd & Doney 2002). Additionally, the supply of nutrients may be affected by changes to ocean currents. For example, model studies have found that nitrate (NO₃⁻) supply to the coastal California ecosystem has increased since 1980 (Jacox et al. 2015); in the model, NO₃⁻ input to the euphotic zone was projected to increase by 80% by the year 2100 due to an increased flux of NO₃⁻ from deep waters (Rykaczewski & Dunne 2010). Such a large increase in NO₃⁻ supply has the potential to influence the community composition of microorganisms and their physiology.

In the case of CO₂, the large increase in atmospheric CO₂ concentrations translates to a substantial change in the availability of dissolved inorganic carbon (DIC) in the
ocean. The present-day concentration of dissolved CO$_2$ in the ocean is ~13 µM, and as the ocean becomes more CO$_2$-saturated, concentrations are expected to more than double causing the pH of the ocean to drop from 8.07 to 7.77 (Beardall et al. 2009; Raven et al. 2005). We know that microorganisms can have differing levels of sensitivity to CO$_2$ and ocean acidification (Riebesell 2004; Doney et al. 2009). It is well documented that calcifying phytoplankton, like coccolithophores, are sensitive to ocean acidification because their laths start dissolving with declining pH (Riebesell et al. 2000; Delille et al. 2005; Kroeker et al. 2013), though non-calcifying microorganisms may also be affected by ocean acidification (Tortell et al. 2002, 2008; Wu et al. 2010; Rost et al. 2008). How a phytoplankton species responds to ocean acidification is likely affected by whether they have carbon concentrating mechanisms (CCMs), which help them concentrate inorganic carbon into their cells. As the partial pressure of ambient CO$_2$ increases, it has been suggested that there will be less of a need for CCMs (Tortell et al. 2008), and that microorganisms will be able to allocate the energy and resources required to maintain CCMs toward other physiological processes (Raven 1991). Likewise, microorganisms that obtain CO$_2$ solely through passive diffusion may also benefit as DIC concentrations increase (Beardall et al. 2009).

If microorganisms are able to take up DIC more effectively, growth rates and the demand for other nutrients, such as nitrogen and phosphorus, will likely increase. The source of these nutrients varies from system to system. In coastal systems, nutrient supply is influenced by terrigenous sources, including riverine discharge or runoff that contains nutrients from wastewater treatment plants and agricultural production. In the past few decades the use of fertilizers made with organic forms of nitrogen, primarily urea, has
increased more than 100-fold (Glibert et al. 2006). The delivery of excess nutrients to the coastal environment is one factor that has the potential to increase the prevalence of harmful algal blooms (HABs). In the last few decades HABs have become a recurring issue off the coast of California (Schnetzer et al. 2007; Anderson et al. 2008; Lewitus et al. 2012), and within the Southern California Bight region, nutrients supplied from natural sources (upwelling) and anthropogenic sources, namely treated wastewater effluent, can be on the same order of magnitude (Howard et al. 2014). One frequent bloomer in the Southern California Bight is *Pseudo-nitzschia* spp., a genus capable of producing domoic acid, the toxin responsible for amnesic shellfish poisoning (Bates et al. 1989; Bates & Trainer 2006; Schroeder et al. 2015). The response of *Pseudo-nitzschia* spp. to organic forms of nitrogen, such as urea, is particularly important to consider as urea can sustain blooms of this species when other sources of nitrogen are in low supply (Cochlan et al. 2008), and certain strains produce more domoic acid when grown on urea than when grown on other nitrogen sources (Howard et al. 2007; Auro & Cochlan 2013; Martin-Jézéquel 2015). Additional and different nutrient substrates, coupled with high temperatures, may create ideal conditions for blooms of *Pseudo-nitzschia*. For example, in 2014 and 2015 the North Pacific, including waters off the coast of southern California, was characterized by unusually warm sea surface temperatures that were as much as 3°C higher than average. The anomalously warm water moved into coastal waters resulting in the largest and most prolonged bloom of *Pseudo-nitzschia* spp. along the west coast of the United States within the past 15 years (Bond et al. 2015; Du et al. 2016; McCabe et al. 2016).

As sea surface temperatures and CO$_2$ concentrations continue to rise, it is likely
that nutrient regimes will shift, new microbial communities will become established (Sarmiento et al. 2010), and rates of nitrogen and carbon uptake will be altered. In this study, a factorial design was used to assess the individual and combined effects of increases in temperature and CO₂ on the rate of NO₃⁻, urea, and DIC uptake by a Southern California Bight microbial community.

MATERIALS AND METHODS

Sample Collection

Seawater was collected from Fish Harbor at the Southern California Marine Institute’s (SCMI) dock in Terminal Island, California (33°44’59” N; 118°12’54” W) on May 10, 2012. Seawater was drawn from just below the surface using a manual suction pump and collected in acid washed (10% HCl) cubitainers that were pre-rinsed with seawater. Collected seawater was transported directly to the University of Southern California (USC) where it was immediately dispensed into 2.7-L polycarbonate bottles and placed within two ecostat continuous culture systems. Diluent, seawater amended with either NO₃⁻ or urea, was continually added to the ecostats over the duration of the 10-day experiment. Samples for this study were collected at 48 h intervals to determine the rate of uptake of two stable isotopically-labeled nitrogen substrates, NO₃⁻ and urea, as well as labeled HCO₃⁻ (referred to as DIC uptake henceforth) by the microbial community. Additional samples were collected at the start and end of the experiment so that changes in nutrient pools could be assessed.
**Ecostat Design**

Ecostats are continuous culture systems that create a controlled experimental setting to culture microorganisms over extended periods of time (Hutchins et al. 2003; Hare et al. 2007; Feng et al. 2009, 2010). Continuous culturing is advantageous because nutrients are delivered at a constant rate, there is quantitative removal of biomass, and the response of microbial communities to changing variables can be examined over extended periods of time (i.e. weeks to months) (Hutchins et al. 2003; Pickell et al. 2009). As a result, the microbial community has sufficient time to acclimate to experimental conditions and competitive displacement will occur, meaning that microorganisms better suited to the experimental conditions will thrive, while those less adapted will have a lower abundance or be lost. One difference between nature and ecostats, however, is that loss processes, such as grazing, can be selective for certain species while cells lost from the ecostat are removed indiscriminately.

In this experiment, temperature, CO$_2$ level, and nutrient inputs were manipulated using two ecostat systems, one for each temperature level, each of which contained 12 individual bottles. The ecostats were set to test present-day (19°C and 380 ppm CO$_2$) and potential future temperature (23°C) and CO$_2$ (800 ppm) conditions. Control (19°C, 380 ppm CO$_2$), +CO$_2$ (19°C, 800 ppm CO$_2$), +Temp (23°C, 380 ppm CO$_2$), and Combined (23°C, 800 ppm CO$_2$) are the terms that will be used to describe the treatments throughout the manuscript.

Each of the treatment bottles in the Control, +CO$_2$, +Temp, and Combined factorial matrix received either NO$_3^-$ or urea as the primary nitrogen source. Seawater used for diluent was collected from Redondo Beach Harbor on May 9, 2012, filtered
using an acid-washed, in-line 0.2 µm cartridge filter, and placed in two 50-L reservoirs. The second collection of water that occurred on May 10, 2012 was necessary because biomass was low in the Redondo Beach Harbor water and could not be used as the inoculant. NO$_3^-$ was added to one reservoir to a final concentration of 15.1 ± 0.6 µmol N L$^{-1}$, and urea was added to the other to a final concentration of 11.1 ± 0.4 µmol N L$^{-1}$ (Table 1). We note that, while the diluent contained these elevated concentrations of NO$_3^-$ and urea, the cells within the ecostats were never exposed to concentrations this high, because the diluent was added slowly over time and the cells were taking the substrates up as it was added. Both diluent reservoirs were amended with silicic acid (H$_4$SiO$_4$) and phosphate (PO$_4^{3-}$) to a final concentration of ~42 µmol Si L$^{-1}$ and ~2 µmol P L$^{-1}$ (Table 1). The concentration of ammonium (NH$_4^+$) in both diluent types was ~2 µmol N L$^{-1}$; we note that this concentration is ~1 µmol N L$^{-1}$ higher than the site water (Table 1), and may be due to contamination that occurred during the experimental set-up. Diluent from each reservoir was supplied to triplicate incubation bottles for each treatment using Teflon lines and adjustable peristaltic pumps. The Teflon lines entered the incubation bottles through the top and extended to the bottom where the diluent was released. Temperature levels were maintained using thermostatically-controlled, recirculating heater/chiller systems set to either 19 or 23°C (Hare et al. 2007, Feng et al. 2009, 2010). The CO$_2$ concentrations within the +CO$_2$ and Combined treatments were manipulated using commercially prepared CO$_2$/air mixtures (Praxair) bubbled into the incubation bottles through Teflon tubes.

The ecostats were positioned on the roof of the Allan Hancock Foundation building so that they would be exposed to ambient light conditions. The incubation
bottles were secured to a plexiglass rack designed to automatically flip on its side every 5 minutes. This motion re-suspends the cells that may have settled on the bottom, and allows the biomass to be homogenously removed through the outflow lines, which are situated on the upper shoulder of the incubation bottles. Consistent, quantitative removal of biomass is key to the ecostat design, as it is this mechanism that allows growth rates of the community to equal loss rates, which enables the conditions within the ecostat to become and remain relatively stable over time. We note that although conditions were generally uniform during the experiment, changes were continuously occurring, which is typical of continuous culture experiments looking at natural microbial assemblages (Macintyre & Cullen 2005; Pickell et al. 2009). The dilution rate was set to 0.3 day\(^{-1}\). This rate was selected based on typical specific growth rates of phytoplankton communities in California coastal waters (Landry et al. 2009; Li et al. 2010). Within the ranges reported in those studies (~0.3–0.6), we conservatively established our dilution rate so we would not risk washing out microorganisms with slower growth rates.

The SCMI community incubated for 24 h in the ecostat bottles before any diluent (NO\(_3^-\) or urea based media) was added. After dilutions began, the first sampling event occurred 24 h later. These steps were completed in sequence to ensure that cells within the ecostats were growing and biomass was increasing before we began measuring rates.

**Nutrient Uptake Experiments**

To determine rates of nitrogen and DIC uptake in the ecostat treatments, an acid washed syringe was used to collect 250 mL from each bottle. Care was taken to only draw off approximately 10% of the bottle volume to avoid substantial disruptions to the continuous culture nutrient inflow/biomass accumulation balance (Hutchins et al. 2003).
Sub-samples were collected to measure concentrations of either NO$_3^-$ or urea, corresponding to the diluent type that the treatment received. The remaining volume was used to measure uptake rates in acid washed (10% HCl with multiple rinses) 230 mL polycarbonate conical bottles. Uptake experiments were performed using two different $^{15}$N-labeled substrates—potassium nitrate (K$^{15}$NO$_3$; 98%) and $^{15}$N-urea (98%; Cambridge Isotope Laboratories, Andover, MA). To measure DIC uptake, $^{13}$C-labeled NaH$^{13}$CO$_3$ (99%; Cambridge Isotope Laboratories) was used and added to all treatments (Hama et al. 1983). We note that the use of NaH$^{13}$CO$_3$ prevented the use of dual $^{15}$N and $^{13}$C-labelled urea, and so uptake of urea-C was not measured; generally uptake of urea-C is very low (Bronk et al. 2014; Bradley et al. 2010a) or below detection (Bradley et al. 2010b). Uptake of NO$_3^-$ was measured in the treatments that received NO$_3^-$-based diluent, while uptake of urea was measured in the treatments that were supported by urea-based diluent. Concentrations of NO$_3^-$ and urea were not known at the start of the $^{15}$N incubations, so additions of 1 µmol N L$^{-1}$ as $^{15}$NO$_3^-$ or $^{15}$N-labeled urea were used. These additions were above tracer level for NO$_3^-$ and urea throughout the experiment. The choice to use a larger addition was made to increase the likelihood that substrate concentrations would be similar across all treatments during the $^{15}$N incubations.

The bottles with added tracer were incubated within the ecostats, under the same temperature and light conditions as the larger ecostat experimental bottles. During the incubations, CO$_2$ was not bubbled into the uptake bottles, however, the uptake bottles were filled to capacity, which minimized head space and CO$_2$ exchange. Bottles were incubated for approximately 3 to 4 h and then filtered sequentially to collect two size fractions (0.7 - 5.0 µm and >5.0 µm). The larger size fraction (>5.0 µm) was collected on
a Sterlitech silver membrane filter. The filtrate was then passed through a Whatman glass fiber filter (GF/F; nominal pore size of 0.7 µm) to collect the smaller size fraction (0.7 - 5.0 µm). Uptake experiments were conducted every other day throughout the duration of the experiment for a total of five time points over 10 days.

**Dissolved and Particulate Nutrient Analyses**

Concentrations of NO$_3^-$ and urea were measured on the same days that uptake experiments were performed. Additional nutrient samples ($NH_4^+$, nitrite (NO$_2^-$), PO$_4^{3-}$, H$_4$SiO$_4$, dissolved primary amines (DPA), total dissolved nitrogen (TDN), dissolved organic carbon (DOC)) were collected at the beginning and end of the experiment so that net changes in specific nutrient pools could be assessed. All nutrient samples were filtered through Whatman GF/F filters that had been combusted for 2 h at 450°C. Samples were stored at -20°C until analysis.

Concentrations of $NH_4^+$ were measured in triplicate using the phenol-hypochlorite method (detection limit (DL) 0.05 µmol N L$^{-1}$, Koroleff 1983) on a Shimadzu UV-1601 spectrophotometer. Concentrations of NO$_3^-$, NO$_2^-$, PO$_4^{3-}$, and H$_4$SiO$_4$ were measured in duplicate on a Lachat QuickChem 8500 autoanalyzer (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; Smith & Bogren 2001; Knapel & Bogren 2001). The manual diacetyl monoxime thiosemicarbazide method was used to analyze the concentration of urea in duplicate (DL 0.025 µmol N L$^{-1}$; Price & Harrison 1987). DPA samples were measured in triplicate using the OPA ($o$-phthaldialdehyde) method followed by analysis on a Shimadzu RF-1501 spectrofluorometer (DL 0.025 µmol N L$^{-1}$; Parsons et al. 1984). TDN and DOC were measured on a Shimadzu 5000A TOC-V/TNM (DL 2 µmol N L$^{-1}$, 5 µmol C L$^{-1}$; Sharp et al. 2004); deep-sea reference
water samples from the University of Miami consensus reference material program were included to ensure analytical accuracy (Hansell 2005). Dissolved organic nitrogen (DON) was calculated by taking the difference between TDN and the sum of the inorganic nitrogen species (NH$_4^+$, NO$_3^-$, and NO$_2^-$); standard deviations for the final DON concentrations were calculated using propagation of error. DIC was measured using coulometry (CM5230, UIC) following King et al. (2011); CO2SYS software (Lewis and Wallace, 1998) was used to calculate pCO$_2$ (in µatm). A Europa 20/20 isotope ratio mass spectrometer was used to analyze particulate nitrogen (PN) and particulate carbon (PC) concentrations and to measure sample isotopic enrichment of $^{15}$N and $^{13}$C.

Samples were collected at the start of the experiment to determine the initial community composition. Cells were preserved using an acidified Lugol’s solution, identified and enumerated according to Tomas (1997) and the Utermöhl method (Utermöhl 1931), respectively, using an inverted compound light-microscopy with an Accu0Scope 3032.

**Uptake Calculations**

Specific ($V$) and absolute ($\rho$) uptake rates of NO$_3^-$ and urea were calculated using equation 2 and 3, respectively, from Dugdale & Wilkerson (1986):

$$ V = \frac{PN \text{ at} \% \times \text{ xs}}{SP \text{ at} \% \times \text{ xs} \times \text{ Time}} $$

$$ \rho = \frac{PN \text{ at} \% \times \text{ xs}}{SP \text{ at} \% \times \text{ xs} \times \text{ Time}} \times [PN] $$

where $V$ is specific uptake rate (h$^{-1}$), and $\rho$ is absolute uptake rate (µmol NL$^{-1}$ h$^{-1}$); PN
at%xs is the $^{15}\text{N}$ atom % enrichment of PN minus 0.366, which is the typical $^{15}\text{N}$ atom % enrichment of an atmospheric $\text{N}_2$ gas standard. SP at%xs is the initial enrichment of the nitrogen Source Pool minus $^{15}\text{N}$ atom % enrichment of the atmospheric standard; in this study the Source Pool was either $\text{NO}_3^-$ or urea. The ambient concentration for $\text{NO}_3^-$ and urea were measured each time an uptake experiment was conducted, and these values were used to calculate the initial enrichment of the nitrogen source pool. $[\text{PN}]$ is the concentration of PN at the end of the incubation ($\mu\text{mol N L}^{-1}$). $V$ expresses the physiological response of the community, and $\rho$ normalizes for differences in biomass between treatments. Specific and absolute uptake rates of DIC were also calculated using the same equations, however, the nitrogen parameters were substituted with carbon, and $PC$ at%xs is the $^{13}\text{C}$ atom % enrichment of PC minus 1.08, which is the natural $^{13}\text{C}$ enrichment of phytoplankton (Slawyk et al. 1977; Lefebvre et al. 2012). DIC concentrations were measured at two time points during the experiment, and the average DIC concentration for each experimental condition (Control, +Temp, +CO$_2$, Combined; $n=12$) was used in the calculation of uptake rates.

**Statistical Analyses**

To calculate average uptake rates and average stoichiometric uptake ratios for the four treatments (Control, +Temp, +CO$_2$, and Combined), all replicates within each treatment were combined and averaged ($n=15$; 3 replicates of each treatment $\times$ 5 time points). While the magnitude of $V$ and $\rho$ varied throughout the experiment, the trends were generally the same across time points. Expressing uptake rates as a combined average of the time points increased variability, but was done to produce greater statistical power.
Data were analyzed using the R statistical program (Team 2010). Data were checked for normality and homogeneity of variance. Data that were not normally distributed were log transformed prior to statistical analysis. A one-way repeated measures analysis of variance (ANOVA) was used to determine if there were significant differences in uptake rates between treatments, nitrogen source (NO$_3^-$ and urea), and size fractions. A post hoc Tukey’s Test was run in order to locate the uptake means that were significantly different from one another. Means were considered to be significantly different if the p value was $\leq 0.05$.

**RESULTS**

*Source Water Characterization and Nutrient Concentrations*

Concentrations of NO$_3^-$, NO$_2^-$, and urea were higher in Redondo Harbor relative to the SCMI dock. Concentrations of NO$_3^-$ and urea, respectively, were 4.30 µmol N L$^{-1}$ and 1.44 µmol N L$^{-1}$ in Redondo Harbor compared to 0.51 µmol N L$^{-1}$ and 0.84 µmol N L$^{-1}$ at the SCMI dock. At both locations, DON made up >50% of the TDN pool, with urea accounting for 25% of the DON pool in Redondo Harbor and 13% of the DON pool at the SCMI dock. The concentration of PO$_4^{3-}$ at Redondo Harbor was double the concentration at the SCMI dock, and the concentration of H$_4$SiO$_4$ was more than triple that seen off the SCMI dock (Table 1).

Every other day during the experiment, concentrations of NO$_3^-$ were measured in the treatments that received NO$_3^-$ diluent and urea in the treatments that received urea diluent (Table 2). Concentrations of NO$_3^-$ were less than 1 µmol N L$^{-1}$ at all time points with the exception of the +CO$_2$ treatment on day 4. Concentrations of urea were
consistently higher than concentrations of NO$_3^-$ during the experiment. The two treatments exposed to elevated temperatures were more efficient at drawing down the available urea in the ecostats (Table 2).

The full suite of nutrients was measured in the ecostat bottles on the final day of the experiment. Nutrient concentrations were generally similar between treatments (Table 1). The concentration of TDN was 5.5-6.5 µmol N L$^{-1}$ in all treatments, with NH$_4^+$ comprising the largest portion of the inorganic nitrogen fraction. NO$_3^-$ concentrations were low, measuring approximately 0.05 µmol N L$^{-1}$ in all treatments, and concentrations of PO$_4^{3-}$ were below the limit of detection (Table 1). Although NO$_3^-$ and PO$_4^{3-}$ concentrations were low or below detection on the final day, both were continually supplied in the seawater diluent at a constant rate throughout the experiment. Concentrations of H$_4$SiO$_4$ and DOC varied between treatments at the end of the experiment. For both diluent types DOC concentrations in the +Temp and Combined treatments were approximately 20 µmol C L$^{-1}$ higher in concentration than the Control and +CO$_2$ treatments.

**Initial Community and Biomass**

Diatoms dominated the initial community that was collected off of the SCMI dock, accounting for 99% of the taxa that were present. Approximately half of the diatom community was comprised of the *Pseudo-nitzschia* genera, including both *Pseudo-nitzschia multiseries* and *Pseudo-nitzschia hasleana*, whose relative abundances were 48% and 4% respectively. Other dominant diatoms included *Leptocylindrus danicus* (39%) and *Chaetoceros* spp. (5%).
PC and PN concentrations of the whole community (> 0.7 µm) were more constant in the treatments receiving NO$_3^-$ diluent, but more variable and higher in the treatments receiving urea diluent (Figure 1).

**Nitrogen Uptake**

Results from the community incubated in the ecostats had two general trends. Uptake rates of NO$_3^-$ (both $V_{NO3}$ and $\rho_{NO3}$) were generally lower than uptake rates of urea (both $V_{UREA}$ and $\rho_{UREA}$), and uptake rates were higher in the larger size fraction (> 5.0 µm) relative to the smaller size fraction (0.7 – 5.0 µm) (Figure 2). Elevated CO$_2$ resulted in higher NO$_3^-$ uptake rates by smaller microorganisms and elevated temperature resulted in higher urea uptake rates by both large and small microorganisms. These general trends were also observed over the course of the experiment (Figure 3). Uptake rates changed the most within the first 2-4 days of the experiment, particularly in the case of urea. After the fourth day uptakes rates remained relatively stable in most treatments (Figure 3).

NO$_3^-$ uptake rates ($V_{NO3}$ and $\rho_{NO3}$) in the larger size fraction did not significantly differ between any of the treatments (Figure 2, p > 0.05). In the +CO$_2$ treatment, however, $V_{NO3}$ and $\rho_{NO3}$ within the smaller size fraction were significantly higher (~50%) than the Control (Figure 2, p < 0.007). Although CO$_2$ was also manipulated in the Combined treatment, no significant increase in NO$_3^-$ uptake was observed in that treatment compared to the Control.

Urea uptake rates ($V_{UREA}$ and $\rho_{UREA}$) showed a distinctly different pattern. For $V_{UREA}$ and $\rho_{UREA}$ in the large size fraction, the +Temp treatment and the Combined treatment were significantly higher than both the Control and the +CO$_2$ treatment.
(Figure 2, p < 0.01). In the smaller size fraction the +Temp and Combined treatments had significantly higher $V_{\text{UREA}}$ than the Control and +CO$_2$ treatment (p < 0.001). The same results were seen for $\rho_{\text{UREA}}$ (p < 0.01) except the Combined treatment did not have significantly higher uptake rates than the +CO$_2$ treatment.

**DIC Uptake**

DIC uptake rates in the larger size fraction were significantly greater than uptake rates in the smaller size fraction for both $V_{\text{DIC}}$ and $\rho_{\text{DIC}}$. This pattern occurred in all experimental treatments (Figure 4, p < 0.001). Higher DIC uptake rates were observed in the + Temp and Combined treatments, following the same general trends observed for urea. In the smaller size fraction this trend was significant for both $V_{\text{DIC}}$ and $\rho_{\text{DIC}}$ when comparing the +Temp treatment to the Control (p < 0.006). The trend was also significant in the smaller size fraction when comparing $V_{\text{DIC}}$ and $\rho_{\text{DIC}}$ of the Combined treatment grown on NO$_3^-$ to the Control (p < 0.001). In the larger size fraction, $\rho_{\text{DIC}}$ of the +Temp treatment grown on NO$_3^-$ had significantly higher rates than the Control, and $\rho_{\text{DIC}}$ of the Combined treatment grown on urea was significantly higher than the Control (p<0.03). None of the +CO$_2$ treatments were significantly different than the Controls, however, $V_{\text{DIC}}$ and $\rho_{\text{DIC}}$ of the larger size fraction in the +CO$_2$ treatments were significantly lower than the +Temp treatments (p < 0.02).

**Stoichiometry**

Particulate concentrations and C:N ratios were impacted by elevated temperature, although the trends were not statistically significant (p > 0.05). Concentrations of PC for the +Temp and Combined treatments were higher than the Control and +CO$_2$ treatments for both size fractions and both diluent types (Table 3). This trend was also found for PN.
concentrations in the treatments that received NO$_3^-$ diluent, but not in the treatments that received urea diluent. For all treatments, PC:PN ratios increased in the +Temp and Combined treatments, while the +CO$_2$ treatment had similar ratios to the Control (Table 3).

Temperature also impacted the stoichiometric uptake ratio of DIC to NO$_3^-$, In the larger size fraction, the ratio of $\rho_{\text{DIC}}:\rho_{\text{NO}_3}$ for the +Temp treatment was significantly higher than the Control (Figure 5, p < 0.05). In the smaller size fraction, the Combined treatment had significantly higher $\rho_{\text{DIC}}:\rho_{\text{NO}_3}$ than the Control (Figure 5, p < 0.02). In contrast, the ratios of $\rho_{\text{DIC}}:\rho_{\text{UREA}}$ in all treatments were not significantly different from the Control.

**DISCUSSION**

The goal of this study was to determine how uptake of NO$_3^-$, urea, and DIC by Southern California Bight microorganisms will be affected by elevated temperature and CO$_2$ conditions that will likely develop over the next century. The SCMI microbial community used to set up the ecostats was dominated by diatom species that are commonly found in coastal southern California waters (Venrick 2015; Reid et al. 1978; Reid et al. 1970; Cullen et al. 1982; Cupp 1943), including *Pseudo-nitzschia* spp., *Leptocylindrus danicus* and *Chaetoceros* spp.. Both diluent types used in this experiment were supplemented with H$_4$SiO$_4$ ensuring that the diatom community would not become H$_4$SiO$_4$ limited. Thus, our study demonstrates how a coastal California microbial assemblage primarily composed of diatoms would be affected by climate change parameters and changes to the dominant nitrogen substrate. At the end of the experiment,
*Leptocylindrus danicus* remained dominant in all treatments, and the relative abundance of other diatom species varied significantly between treatments (Tatters et al. in prep).

**Nutrient Uptake in the +Temp Treatment**

Many studies document that a temperature increase of a few degrees increases microbial growth rates and photosynthesis (Eppley 1972; Geider 1987; Raven & Geider 1988; Davison 1991; Lomas & Glibert 1999; Berges et al. 2002; Boyd et al. 2013). Studies using both cultured phytoplankton and field communities find that the relationship between temperature and nitrogen uptake varies between species (Goldman 1977; Kristiansen 1983; Glibert et al. 1995; Lomas & Glibert 1999). Of these studies Lomas & Glibert (1999) is the most similar to our study, as they use $^{15}$N incorporation to measure both NO$_3^-$ and urea uptake by diatom-dominated field communities subjected to temperature manipulations that were similar to the temperatures used in our study. In their study, for every 1°C change in temperature, NO$_3^-$ uptake decreased by 2.9% on average. We found that temperature did not impact NO$_3^-$ uptake, but consistent with Lomas & Glibert (1999), temperature did significantly increase uptake of urea (Figure 2).

There are a few possible explanations for why temperature did not significantly affect NO$_3^-$ uptake in our study. Seawater was collected from the surface close to shore, so microorganisms in this region may have already been accustomed to temperature fluctuations of several degrees. The amplitude of sea surface seasonal variation off the coast of Southern California can reach 3.1°C (Nezlin et al. 2004). Thus, our temperature increase of 4°C is close to the realm of natural seasonal variation. It is also possible that the ambient (19°C) and elevated (23°C) temperatures within this study were above the optimal physiological range for diatom NO$_3^-$ uptake. In the diatom Skeletonema
*costatum* that typically inhabits seawater that is 12-15°C the activity of nitrate reductase (NR) is unstable above ~16°C (Gao et al. 2000). Likewise, Berges et al. (2002) show $^{15}$N uptake and NR activity of a temperate diatom, *Thalassiosira pseudonana*, declines between 17 and 25°C. The optimal temperature range for growth of *Leptocylindrus danicus*, one of dominant phytoplankton species in this study, is between 15 and 20°C (Verity 1982), so it is possible that NO$_3^-$ uptake physiology falls in this range as well.

In the case of urea, our data show a positive relationship between temperature and urea uptake. The majority of studies that measure temperature effects on urea uptake measure the activity and expression of enzymes, like urease, involved in urea metabolism rather than directly quantifying urea uptake. These studies show that urease activity (Fan et al. 2003) and urea uptake (Lomas & Glibert 1999) are positively correlated with temperature. In our study raising the temperature by 4°C increased uptake rates of urea ($V_{UREA}$) by 80% for small microorganisms and 54% for large microorganisms. The substantial increase in urea uptake rates suggest that as sea surface temperatures continue to rise, organisms capable of using urea may have an advantage, especially in temperate coastal systems that receive wastewater effluent or agricultural runoff, both of which contain urea (Bronk et al. 2010; Howard et al. 2014). Urea is also the most common form of nitrogen used in fertilizers for agricultural production (Merigout et al. 2008; Glibert et al. 2006), and California is the top agricultural center in the United States (California Department of Food and Agriculture 2015). With respect to the Southern California Bight, ~60% of the total nitrogen in riverine runoff is comprised of organic forms, including urea, and riverine runoff has been identified as the primary source of urea to this system (Howard et al. 2014). Urea is a small component, however, of the
total nitrogen load delivered to the Southern California Bight when both natural and anthropogenic sources are included.

Similar to urea, there was also an increase in DIC uptake with increasing temperature (Figure 4). This trend occurred regardless of the nitrogen substrate added to the diluent. The general model is that increased temperature elevates carbon fixation and respiration up to a certain threshold that is species-specific and contingent upon the organism’s geographic location and inherent ability to acclimate (Eppley 1972; Goldman 1977; Verity 1982; Thomas et al. 2012; Boyd et al. 2013). In the past decade more work has been done on communities rather than cultures, and the relationship between temperature and carbon consumption is proving to be more complex. A few studies even show that elevated temperature decreases carbon drawdown (Wohlers et al. 2009; Kim et al. 2011), which is contrary to our results. Not only did DIC uptake rates increase as a function of temperature, but concentrations of PC and DOC also increased in the +Temp treatments (Tables 1 and 4). Our findings resemble the results reported in Taucher et al. (2012), where a Baltic Sea microbial community was grown for a month at a range of temperatures resulting in a net increase of particulate and dissolved carbon. They hypothesize that increased temperatures may lead to carbon overconsumption and a decoupling between C:N ratios in both the particulate and dissolved form, leading to a build up of particulate and dissolved carbon and an increase in C:N ratios. This hypothesis is also supported by our study where the PC:PN ratios (Table 3) and the ratio of absolute DIC uptake to absolute NO$_3^-$ uptake were both elevated in the +Temp treatment (Figure 5). The average ratio of $\rho_{\text{DIC}}/\rho_{\text{NO}_3^{-}}$ was significantly higher in the + Temp treatment compared to the Control for the larger size fraction. Higher DIC to NO$_3^-$
uptake ratios at elevated temperature may be linked to the presence of diatoms. In a culture experiment the diatom *Thalassiosira weissflogii* had higher DIC to NO$_3^-$ uptake ratios when temperature was elevated by 5°C (Taucher et al. 2015). In this same study, however, the opposite pattern occurred for the diatom *Dactyliosolen fragilissimus*. This finding warrants further investigation because of the implications for shaping ocean carbon biogeochemistry and C:N stoichiometry in the future.

**Nutrient Uptake in the +CO$_2$ Treatment**

The physiological effects that CO$_2$ has on the uptake of nutrients are complex. Elevated CO$_2$ can significantly impact enzyme activity and subsequent metabolic processes such as photosynthesis (Mercado et al. 1999; Zou et al. 2011), calcification (Doney et al. 2009 and references therein), and nutrient uptake and assimilation (Magnusson et al. 1996; Gordillo et al. 2001; Zou 2005; Hofmann et al. 2013). Uptake of nitrogen is intrinsically linked to carbon utilization, and the mechanism by which microorganisms use carbon varies between species. Some microorganisms contain CCMs, and those that do not rely on passive diffusion to acquire CO$_2$ (Raven 1991; Kaplan & Reinhold 1999; Giordano et al. 2005). Cells that actively use CCMs to acquire CO$_2$/and or HCO$_3^-$ are rendered less ‘leaky’ when surrounded by seawater with higher CO$_2$ concentrations, causing the regulatory enzymes that operate the CCM to be down-regulated resulting in more energy that can be allocated to support physiological processes such as growth and nutrient assimilation (Burkhardt et al. 2001; Rost et al. 2003). Production and growth of species that rely on passive diffusion should be enhanced by higher ambient CO$_2$ concentrations. The metabolic processes of microorganisms without CCMs are more likely to be enhanced when CO$_2$ is increased.
than those microorganisms with CCMs (Giordano et al. 2005). Under elevated CO₂ conditions community shifts can occur depending on the effectiveness of the mechanism that microorganisms use to acquire inorganic carbon (Tortell et al. 2002; Tortell et al. 2008; Feng et al. 2009, 2010; Hare et al. 2007), translating to differences in uptake rates of other nutrients. In our study, elevated CO₂ concentrations influenced the uptake of NO₃⁻, while urea uptake was unaffected (Figure 2).

Although few studies have examined how NO₃⁻ uptake by microalgae is affected by CO₂ concentration, the response appears to differ between microbial groups. We found this to be true in our study, given that uptake rates of NO₃⁻ by smaller microorganisms were significantly higher at elevated CO₂, while uptake rates of NO₃⁻ by larger microorganisms increased but not significantly. Consistent with our results for smaller microorganisms, Beardall & Koss (unpublished data described in Beardall et al. 2009) show that uptake of nitrogen was enhanced by elevated CO₂ concentrations in species of phytoplankton; however, the nitrogen substrate and phytoplankton group(s) that they used were not identified. Other mesocosm studies, however, show that uptake of NO₃⁻ is unaffected by varying levels of CO₂ in communities where diatoms were present (Riebesell et al. 2007) and absent (Engel et al. 2005).

Like urea, the uptake of DIC in both nitrogen diluent types and both size fractions was unaffected under high CO₂, contrary to several studies (Trimborn et al. 2009; Hutchins et al. 2007). The coastal California ecosystem is an upwelling system characterized by high levels of DIC and low pH (Hauri et al. 2009) with a high degree of temporal variability (Capone and Hutchins 2013; Leinweber & Gruber 2013) relative to other oceanic regions, making it likely that the microorganisms in our study have already
optimized for high CO$_2$ conditions. Diatoms in particular may be less susceptible to high CO$_2$ conditions than other microalgae groups because of their efficient CCMs (Tortell et al. 2002; Fabry et al. 2008).

**Nutrient Uptake in the Combined Treatment**

We did not detect a significant additive effect of temperature combined with CO$_2$ on the uptake of NO$_3^-$, urea, or DIC. Even though $V_{\text{UREA}}$ and $\rho_{\text{UREA}}$ were significantly higher than the Control in the Combined treatment, this was likely a function of temperature, as the combined effect was not significantly greater than the impact of temperature alone and urea uptake was unaffected by elevated CO$_2$. Our results for DIC uptake in the Combined treatment were also likely driven by temperature. It is interesting that NO$_3^-$ uptake rates by smaller microorganisms in the Combined treatment were not significantly higher than the Control, since rates significantly increased when CO$_2$ alone was manipulated. The smaller microorganisms in the Combined treatment may have preferred other nitrogen substrates (i.e. urea) when both temperature and CO$_2$ were elevated.

The results for nitrogen and carbon uptake in the Combined treatment indicate that temperature, more so than CO$_2$, has the greater potential to change the cycling of urea and DIC in California coastal waters in the future. Uptake of NO$_3^-$ may increase as a function of elevated CO$_2$, however, this relationship may only be important during the winter when temperatures are cooler. Our results for dissolved and particulate carbon as well as $\rho_{\text{DIC}}:\rho_{\text{NO3}}$ ratios in the Combined treatment further suggest that C:N ratios are likely to be different under future climate scenarios.


**Nitrate versus Urea**

Within the same experimental conditions uptake rates for urea were significantly greater than uptake rates for NO$_3^-$ (Figures 2 and 3). This result was somewhat counterintuitive given that dissolved concentrations of NO$_3^-$ were consistently lower than urea (Table 1 and 2). One possible explanation is active urea regeneration, which would result in higher urea concentrations than if just uptake was taking place. Regeneration of urea can occur on the order of minutes to days (Bronk et al. 2007), and rates of regeneration can be high in natural waters (Bronk et al 1998). Sources of water column urea regeneration include zooplankton, bacteria, and phytoplankton (reviewed in Solomon et al. 2010). With respect to phytoplankton, some diatoms have been shown to possess the genes that are necessary to produce urea within their cells (Ambrust et al. 2004; Bowler et al. 2008). It is definitely possible that urea was being rapidly used and regenerated within the ecostats.

Kinetics and different half saturating constants ($K_s$) for NO$_3^-$ and urea uptake might also explain why urea uptake was higher than NO$_3^-$ uptake even though dissolved concentrations of NO$_3^-$ were lower than urea. For genera that were present in this study, including *Leptocylindrus* spp. and *Pseudo-nitzschia* spp., $K_s$ values for NO$_3^-$ uptake typically fall between 1 and 3 µmol N L$^{-1}$ (Eppley et al. 1969; Cochlan et al. 2008; Seeyave et al. 2009). Less is known about urea uptake kinetics, but $K_s$ values for diatom urea uptake can range considerably (Bronk & Flynn 2006 and references therein; Solomon et al. 2010 and references therein), falling lower and higher than $K_s$ values for NO$_3^-$ uptake. Cochlan et al. (2008) found that $K_s$ values for NO$_3^-$ uptake by *Pseudo-nitzschia australis* were lower than $K_s$ values for uptake of other nitrogen substrates (NH$_4^+$...
and glutamine), and that urea uptake did not follow typical Michaelis-Menten kinetics because uptake was non-saturating. They suggest that some species of diatoms have a relatively higher affinity for NO$_3^-$, and are, therefore, able to more effectively take it up at low concentrations. In our study, it is possible that the microorganisms were taking up urea more quickly, and yet were not as efficient at drawing urea down to the very low concentrations observed for NO$_3^-$. 

We chose to assess NO$_3^-$ and urea utilization in this study; however, another substrate of interest is NH$_4^+$. Although the microorganisms in the ecostat bottles received considerably less NH$_4^+$ than either NO$_3^-$ or urea, which were added to the diluent, concentrations of NH$_4^+$ were higher than concentrations of both NO$_3^-$ and urea at the SCMI site (Table 1). Given the reduced nature of NH$_4^+$, this substrate is often thought of as the preferred substrate for phytoplankton nitrogen uptake (McCarthy 1981; Glibert et al. 2016 and references therein), and it can inhibit and/or reduce NO$_3^-$ uptake by phytoplankton depending on a variety of factors including ambient concentrations, environmental conditions, and species composition (Dortch 1990). In our study, it is possible that NO$_3^-$ uptake rates were reduced due to the presence of NH$_4^+$, however, any inhibitory effects were likely minimal. First, concentrations of NH$_4^+$ would be low in the ecostats themselves as the diluent was added slowly. Lack of NH$_4^+$ inhibition can also be inferred by the continually low NO$_3^-$ concentrations observed throughout the experiment (Table 2), indicating that NO$_3^-$ was readily being used. It would be useful to include NH$_4^+$ as a substrate in future ecostats, because of the dynamic interactions that occur between NO$_3^-$, NH$_4^+$, and urea with respect to microbial utilization and availability. For example, concentrations of NH$_4^+$ can also increase as urea undergoes hydrolysis.
(Solomon et al. 2010). It would also be worthwhile to repeat the study and measure urea uptake in the treatments that received NO$_3^-$ diluent and vice versa. We were unable to do this due to water limitations; however, background concentrations of both NO$_3^-$ and urea were present in the diluent, and the low concentrations of both NO$_3^-$ and urea measured at the end of the experiment in all treatments, regardless of the diluent type, indicate that the microorganisms in our study were using all of these nutrients.

In the Southern California Bight, it is particularly important to consider possible future changes in the nature and magnitude of nitrogen utilization by diatoms. The production of toxins by HAB species, such as the diatom *Pseudo-nitzschia* that frequently bloom off the coast of California, is influenced by nutrient availability. *Pseudo-nitzschia* is capable of utilizing both inorganic and organic forms of nitrogen, including urea, as a source for growth (Cochlan et al. 2008; Loureiro et al. 2009; Auro & Cochlan 2013), and several factors including nutrient limitation, nitrogen source, and CO$_2$ can influence domoic acid production (Pan et al. 1998; Bates et al. 1993; Bates & Trainer 2006 and references therein; Sun et al. 2011; Sahraoui et al. 2012; Tatters et al. 2012). In this study, *Pseudo-nitzschia* was present throughout the experiment and domoic acid was detected in all treatments, with varying cellular toxin levels (Tatters et al. in prep).

**CONCLUSIONS**

Here we provide evidence that uptake of NO$_3^-$, urea, and DIC is enhanced by changes in temperature and/or CO$_2$. Our results suggest that uptake of urea and DIC by phytoplankton communities dominated by diatoms will increase as sea surface temperatures rise in temperate near shore environments. Although smaller
microorganisms were a minor component of the community that we studied, we found that rates of NO$_3^-$ uptake by smaller microorganisms increase as a function of elevated CO$_2$ concentration. This relationship may be even more important during the winter, or if elevations in temperature and CO$_2$ are decoupled in the future, or when smaller microorganisms are more dominant. The relationship that we found between elevated temperature and urea uptake rates indicate that urea will be more rapidly utilized if warming continues. Additionally, the increased DIC to NO$_3^-$ uptake ratios that occurred at elevated temperature indicates that C:N stoichiometry may also be altered in the future. Finally, this study demonstrates that uptake trends of nitrogen substrates under climate change conditions cannot be generalized, and it is important to study multiple substrates to gain a more comprehensive view of potential change.

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Table 1) Nutrient concentrations of the seawater collected off the Southern California Marine Institute (SCMI) dock and in Redondo Harbor, as well as the two diluent types (NO$_3^-$ and urea-based). Nutrient concentrations measured on the final day of the experiment for each treatment are displayed below the respective diluent type that the treatment received. Concentrations are in units of µmol C, N, P, or Si L$^{-1}$ ± 1 standard deviation (n=3). NM=Not Measured. BD=Below Detection. *Indicates n=2 and error is half the range.

<table>
<thead>
<tr>
<th>Source</th>
<th>DIC µmol C L$^{-1}$</th>
<th>DOC µmol C L$^{-1}$</th>
<th>NH$_4^+$ µmol N L$^{-1}$</th>
<th>NO$_2^-$ µmol N L$^{-1}$</th>
<th>NO$_3^-$ µmol N L$^{-1}$</th>
<th>DON µmol N L$^{-1}$</th>
<th>Urea µmol N L$^{-1}$</th>
<th>DPA µmol P L$^{-1}$</th>
<th>PO$_4^{3-}$ µmol P L$^{-1}$</th>
<th>H$_2$SiO$_4$ µmol Si L$^{-1}$</th>
<th>% NH$_4^+$</th>
<th>% NO$_3^-$</th>
<th>% Urea</th>
<th>% DON</th>
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<td>SCMI (starting community)</td>
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<tr>
<td>Control</td>
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<td>113 ± 1</td>
<td>0.16 ± 0.0</td>
<td>0.19 ± 0.0</td>
<td>15.10 ± 0.6</td>
<td>5.67 ± 2.3</td>
<td>1.22 ± 0.0</td>
<td>0.09 ± 0.0</td>
<td>2.17 ± 0.2</td>
<td>41.9 ± 1.7</td>
<td>8.3</td>
<td>66.0</td>
<td>5.3</td>
<td>24.9</td>
</tr>
<tr>
<td>Combined</td>
<td>2188 ± 64</td>
<td>156 ± 11</td>
<td>0.15 ± 0.0</td>
<td>0.19 ± 0.0</td>
<td>15.10 ± 0.6</td>
<td>5.67 ± 2.3</td>
<td>1.22 ± 0.0</td>
<td>0.09 ± 0.0</td>
<td>2.17 ± 0.2</td>
<td>41.9 ± 1.7</td>
<td>8.3</td>
<td>66.0</td>
<td>5.3</td>
<td>24.9</td>
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<tr>
<td>Control</td>
<td>2133 ± 42</td>
<td>117 ± 11</td>
<td>0.15 ± 0.0</td>
<td>0.19 ± 0.0</td>
<td>15.10 ± 0.6</td>
<td>5.67 ± 2.3</td>
<td>1.22 ± 0.0</td>
<td>0.09 ± 0.0</td>
<td>2.17 ± 0.2</td>
<td>41.9 ± 1.7</td>
<td>8.3</td>
<td>66.0</td>
<td>5.3</td>
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<tr>
<td>+Temp</td>
<td>2052 ± 49</td>
<td>132 ± 6</td>
<td>0.18 ± 0.1</td>
<td>0.19 ± 0.0</td>
<td>15.10 ± 0.6</td>
<td>5.67 ± 2.3</td>
<td>1.22 ± 0.0</td>
<td>0.09 ± 0.0</td>
<td>2.17 ± 0.2</td>
<td>41.9 ± 1.7</td>
<td>8.3</td>
<td>66.0</td>
<td>5.3</td>
<td>24.9</td>
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<tr>
<td>+CO$_2$</td>
<td>2156 ± 139</td>
<td>112 ± 7</td>
<td>0.10 ± 0.0</td>
<td>0.19 ± 0.0</td>
<td>15.10 ± 0.6</td>
<td>5.67 ± 2.3</td>
<td>1.22 ± 0.0</td>
<td>0.09 ± 0.0</td>
<td>2.17 ± 0.2</td>
<td>41.9 ± 1.7</td>
<td>8.3</td>
<td>66.0</td>
<td>5.3</td>
<td>24.9</td>
</tr>
<tr>
<td>Combined</td>
<td>2162 ± 18</td>
<td>150 ± 22</td>
<td>0.21 ± 0.1</td>
<td>0.19 ± 0.0</td>
<td>15.10 ± 0.6</td>
<td>5.67 ± 2.3</td>
<td>1.22 ± 0.0</td>
<td>0.09 ± 0.0</td>
<td>2.17 ± 0.2</td>
<td>41.9 ± 1.7</td>
<td>8.3</td>
<td>66.0</td>
<td>5.3</td>
<td>24.9</td>
</tr>
</tbody>
</table>
Table 2) NO$_3^-$ and urea concentrations measured on days 2, 4, 6, 8, and 10 for the different treatments. NO$_3^-$ concentrations are from the treatments that received NO$_3^-$ diluent, and urea concentrations are from the treatments that received urea diluent. Concentrations are in units of µmol N L$^{-1}$ ± 1 standard deviation (n=3). *There was an issue with the diluent pump prior to sampling.

<table>
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<tr>
<th>Day</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
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<tr>
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<td>0.07 ± 0.0</td>
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<td>+CO$_2$</td>
<td>0.25 ± 0.1</td>
<td>2.74 ± 3.1*</td>
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<td></td>
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<td>0.35 ± 0.1</td>
<td>0.08 ± 0.0</td>
<td>0.14 ± 0.1</td>
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<td>Urea Concentrations</td>
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<tr>
<td></td>
<td>Control</td>
<td>5.08 ± 0.5</td>
<td>4.51 ± 3.2</td>
<td>3.05 ± 2.3</td>
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<td>+Temp</td>
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<td>1.15 ± 0.9</td>
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<td>+CO$_2$</td>
<td>4.91 ± 0.7</td>
<td>4.60 ± 2.5</td>
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<td></td>
<td>Combined</td>
<td>0.43 ± 0.2</td>
<td>0.52 ± 0.1</td>
<td>0.42 ± 0.1</td>
<td>0.44 ± 0.2</td>
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Table 3) Average particulate carbon (PC) and particulate nitrogen (PN) concentrations and PC:PN ratios for two size classes of microorganisms (0.7-5.0 µm and >5.0 µm) at the Southern California Marine Institute (SCMI) dock and in the different treatments. PC and PN concentrations are in units of µmol C or N L⁻¹ ± 1 standard deviation (n=15; 3 reps × 5 time points).

<table>
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<tr>
<th></th>
<th>0.7-5.0 µm</th>
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<td>PC (µmol C L⁻¹)</td>
<td>PN (µmol N L⁻¹)</td>
<td>PC:PN</td>
<td>PC (µmol C L⁻¹)</td>
<td>PN (µmol N L⁻¹)</td>
<td>PC:PN</td>
</tr>
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<td>SCMI (starting community)</td>
<td>29.0 ± 1.1</td>
<td>3.38 ± 0.2</td>
<td>8.60 ± 0.6</td>
<td>36.6 ± 1.6</td>
<td>3.97 ± 0.1</td>
<td>9.18 ± 0.2</td>
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<td>NO₃⁻ diluent</td>
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<td></td>
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</tr>
<tr>
<td>Control</td>
<td>35.6 ± 6.1</td>
<td>3.72 ± 1.1</td>
<td>10.2 ± 2.3</td>
<td>153.6 ± 50</td>
<td>11.0 ± 2.3</td>
<td>14.2 ± 4.7</td>
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<td>+Temp</td>
<td>39.9 ± 4.8</td>
<td>3.81 ± 0.9</td>
<td>10.8 ± 1.7</td>
<td>208.2 ± 50</td>
<td>13.5 ± 3.1</td>
<td>15.8 ± 3.5</td>
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<tr>
<td>+CO₂</td>
<td>37.4 ± 7.6</td>
<td>3.96 ± 0.8</td>
<td>9.6 ± 1.4</td>
<td>132.5 ± 46</td>
<td>10.2 ± 2.4</td>
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<tr>
<td>Combined</td>
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<td>11.3 ± 1.5</td>
<td>210.2 ± 73</td>
<td>13.9 ± 5.0</td>
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</tr>
<tr>
<td>Control</td>
<td>28.7 ± 7.3</td>
<td>3.32 ± 1.1</td>
<td>9.0 ± 1.6</td>
<td>181.0 ± 100</td>
<td>13.7 ± 4.2</td>
<td>12.5 ± 3.8</td>
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<td>3.23 ± 1.0</td>
<td>10.3 ± 1.3</td>
<td>205.3 ± 51</td>
<td>14.7 ± 2.1</td>
<td>13.9 ± 2.7</td>
</tr>
<tr>
<td>+CO₂</td>
<td>29.6 ± 6.9</td>
<td>3.54 ± 1.2</td>
<td>8.8 ± 1.9</td>
<td>196.3 ± 106</td>
<td>15.5 ± 4.7</td>
<td>12.0 ± 3.7</td>
</tr>
<tr>
<td>Combined</td>
<td>35.9 ± 9.6</td>
<td>3.43 ± 1.2</td>
<td>10.7 ± 1.6</td>
<td>260.6 ± 78</td>
<td>19.0 ± 4.7</td>
<td>13.9 ± 3.9</td>
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</table>
Figure 1) Mean PC (A) and PN (B) concentrations ± 1 standard deviation (n=3) of NO$_3^-$ diluent treatments (shaded markers) and urea diluent treatments (white markers) of the whole community (>0.7 µm) over the course of the experiment.
Figure 2) Mean specific (A) and absolute (B) uptake rates ± 1 standard deviation (n=15) of NO$_3^-$ (solid bars) and urea (dotted bars) by small (0.7-5.0 µm, black bars) and large (>5.0 µm, white bars) microorganisms in the incubated community. Bars are an average of all treatment replicates across 5 time points. Bars that have different letters are significantly different from one another (p ≤ 0.05).
Figure 3) Mean specific (A and B) and absolute (C and D) uptake rates ± 1 standard deviation (n=3) of NO$_3^-$ (shaded markers) and urea (white markers) by small (0.7-5.0 µm, A and C) and large (>5.0 µm, B and D) microorganisms in the incubated community over the course of the experiment. Panel C and D have a different y-axis scale due to large differences in uptake rates between the small and large size fraction.
**Figure 4** Mean specific (A) and absolute (B) uptake rates ± 1 standard deviation (n=15) of DIC by microorganisms sustained on NO$_3^-$ based diluent (solid bars) and urea based diluent (dotted bars) by small (0.7-5.0 µm, black bars) and large (>5.0 µm, white bars) microorganisms in the incubated community. Bars are an average of all treatment replicates across 5 time points. Bars that have different letters are significantly different from one another (p ≤ 0.05).
Figure 5) Stoichiometric ratio of $\rho_{\text{DIC}}$ to $\rho_{\text{NO}_3}$ (solid bars) and $\rho_{\text{DIC}}$ to $\rho_{\text{UREA}}$ (dotted bars) ± 1 SD (n=15) for small (0.7-5.0 µm, black bars) and large (>5.0 µm, white bars) microorganisms in the incubated community. Bars that have different letters are significantly different from one another ($p \leq 0.05$).
CHAPTER 3

IMPACT OF TEMPERATURE, CO₂, AND IRON ON NUTRIENT UPTAKE BY A LATE-SEASON MICROBIAL COMMUNITY FROM THE ROSS SEA, ANTARCTICA

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⁵Integrative Oceanography Division, Scripps Institution of Oceanography, UC San Diego, La Jolla, CA 92037, USA
ABSTRACT: The Southern Ocean is rapidly changing as a result of rising sea surface temperatures, elevated concentrations of CO₂, and modifications to iron sources and sinks. Because the Southern Ocean has seasonally high rates of primary production, it is critical to determine how biogeochemical rate processes and the structure and function of microbial communities will be impacted by these changes in the future. During the 2013 austral summer, we measured rates of nitrogen and carbon uptake by a late-season Ross Sea microbial community under different potential climate change conditions. A natural microbial assemblage was collected from the ice edge in McMurdo Sound, and cultured for nearly a month using both a semi-continuous culturing and continuous culturing ‘ecostat’ approach. The individual and combined impacts of temperature elevation and iron addition were tested during both experiments, and CO₂ level was an additional parameter that was manipulated during the continuous experiment. Nutrient concentrations and biomass parameters were measured during both experiments. During the continuous experiment we also measured uptake rates of nitrate (NO₃⁻) and dissolved inorganic carbon (DIC) by two different size classes (0.7 - 5.0 µm and > 5.0 µm) of microorganisms. Of the three parameters tested, temperature elevation had the largest impact on the microbial community. We found that NO₃⁻ and DIC uptake rates by larger microorganisms significantly increased when temperature was elevated, and that larger microorganisms were more impacted by temperature elevation than smaller microorganisms. Iron addition was also important; however, the magnitude of its impact was greater when temperature was also changed. Our results indicate that NO₃⁻ and DIC
uptake rates may increase in the next century if predictions for sea surface warming in the Southern Ocean are realized. These findings are important because of the implications that they have for estimating new production and potential carbon export to the deep sea.

**INTRODUCTION**

It is well established that the Polar Regions are highly sensitive to global change (IPCC 2014). Substantial alterations have already been witnessed in the Southern Ocean over the past few decades, including atmospheric warming and rising sea surface temperatures (SST), higher concentrations of CO$_2$ in both the atmosphere and ocean, and significant loss of sea ice (Constable et al. 2014, and references therein). The Southern Ocean is large, encompassing ~6.1% of total ocean surface area, and these changes are not occurring at the same rate throughout the region. The greatest changes have occurred along the Western Antarctic Peninsula, where extensive sea ice loss and shifts in biological communities have already been documented (Montes-Hugo et al. 2009). In contrast, the Ross Sea has cooled in recent years, increasing the extent and duration of the sea ice (Comiso et al. 2011; Smith et al. 2012; Stammerjohn et al. 2012). Despite the current cooling trend, the Ross Sea is predicted to warm by 2 - 4°C by the end of the century (Bracegirdle & Stephenson 2012). Although the Southern Ocean has been the focus of a considerable amount of climate change research, we currently do not have a comprehensive understanding nor an ability to predict how this ocean ecosystem will be impacted by anticipated conditions at the end of the century.

It is essential to understand how climate change will impact the Southern Ocean, because it is one of the most biologically productive locations on our planet. The Ross
Sea in particular is among the most productive Antarctic regions, accounting for more than one third of the total production that occurs in Southern Ocean shelf waters (Arrigo et al. 2008). About half of the primary production in the Ross Sea is exported to depth; hence, this region plays a key role in the Southern Ocean carbon cycle (Asper & Smith 2003). As newly fixed carbon sinks from the surface layer, it has the potential to become entrained within the deep ocean circulation system and stored for hundreds of years (Broecker 1991; Sabine et al. 2004). This active biological pump coupled with high CO$_2$ solubility due to cold water (i.e., the solubility pump) makes this system an important global CO$_2$ sink, and studies have found that the Southern Ocean as a whole accounts for about one third of the oceanic uptake of atmospheric CO$_2$ (Mikaloff Fletcher et al. 2006; Gruber et al. 2009). As atmospheric CO$_2$ concentrations continue to rise, regions within the Southern Ocean that have high rates of annual primary production, like the Ross Sea, may become increasingly important in mediating the exchange of CO$_2$ between the ocean and the atmosphere (Arrigo et al. 2008).

Although primary production can be high, the majority of the Southern Ocean has extremely low concentrations of iron (Fe) and is, therefore, considered to be a high nutrient low chlorophyll (HNLC) region. Because Fe is the limiting factor for primary production, questions regarding how the Southern Ocean will respond to increasing SST and elevated CO$_2$ levels are centered around Fe and its availability. Sources of Fe are expected to shift due to the effects of climate change, likely resulting in more Fe supplied to certain regions of the Southern Ocean, particularly those that are influenced by ice (Sedwick and DiTullio 2007). In the Ross Sea, meltwater from glaciers and icebergs are expected to deliver bioavailable Fe to the sea (Raiswell et al. 2008). More Fe may also
be delivered via eolian transport as retreating snow cover on the Antarctic continent exposes more soil to the wind (Jacobs et al. 2002), and as the delivery of eolian dust from other continents, such as Australia, is modified due to shifting wind patterns (Revel-Rolland et al. 2006). Fe may also become more bioavailable due to changes in speciation that are associated with ocean acidification (Stockdale et al. 2016).

Understanding how Southern Ocean phytoplankton, individually and as communities, respond to sea surface warming, elevated CO$_2$ levels, and higher concentrations of Fe will help us to infer how primary production, nutrient cycles, and carbon export may change in the future. Certain species and/or groups of phytoplankton may be at a competitive (dis)advantage in a changing environment due to their (in)ability to physiologically acclimate. In the Southern Ocean, a diverse microbial community exists, consisting of centric diatoms, pennate diatoms, haptophytes, dinoflagellates, and pico- and nano-phytoplankton (Arrigo et al. 1999; Wolf et al. 2013; Wright et al. 2010). Recent studies from the Southern Ocean find that temperature is one of the strongest predictors of diatom primary production (Boyd et al. 2016) and that growth rates of diatoms increase as a function of temperature, while growth rates of *Phaeocystis antarctica*, a haptophyte, remain relatively unchanged (Zhu et al. 2016). Additionally, results from community experiments find that diatoms outcompete *Phaeocystis antarctica* when grown under simulated future conditions that have included temperature elevation as an experimental variable (Rose et al. 2009, Xu et al. 2014). A likely explanation for this is the observation that distinct phytoplankton species display variation in both their thermal tolerance range (i.e., temperature range where growth rate is positive) and their optimum temperature for growth (Thomas et al. 2012).
Along these same lines, some species of phytoplankton are better adapted to live in a high CO₂ ocean that is more acidic (Riebesell 2004; Doney et al. 2009). Because CO₂ has a low affinity for RubisCO, the enzyme at the center of photosynthetic carbon fixation, most phytoplankton utilize carbon concentrating mechanisms (CCMs) to concentrate DIC into their cells and to help them maintain high photosynthetic rates (Colman and Rotatore 1995; Reinfelder 2011). Phytoplankton species employ different CCM strategies that have varying energy requirements (Reinfelder et al. 2000; Raven et al. 2014). As the partial pressure of CO₂ increases, energy expended to operate CCMs will, in effect, be saved, allowing that energy to be allocated toward other cellular functions. Studies conducted using Southern Ocean phytoplankton find that down-regulation of CCM activity under high CO₂ conditions can lead to increased primary production (Tortell et al. 2008, 2010; McMinn et al. 2014), while other studies find that cellular physiology, including growth rates, are not impacted when CO₂ levels are high (Boelen et al. 2011; Crawfurd et al. 2011; Young et al. 2015a). Studies also show that high CO₂ incubations of Ross Sea phytoplankton favor certain species. High CO₂ conditions favored *Chaetoceros lineola*, a large centric diatom, over *Cylindrotheca closterium*, a relatively smaller pennate diatom, in a continuous culture experiment conducted in the Ross Sea (Feng et al. 2010). Differences in phytoplankton community structure associated with CO₂ level were also found by Hoppe et al. (2013), where the relative abundance of *Chaetoceros* sp. increased when CO₂ levels were high.

Although temperature and CO₂ have a clear impact on phytoplankton physiology, it is necessary to consider Fe to attain a more complete understanding of how Southern Ocean phytoplankton will be affected by changing conditions in the future.
Microorganisms in the Southern Ocean are in constant competition for bioavailable Fe; accordingly, they have evolved various strategies, such as reduced cell size, high affinity transporters, and enzyme replacement, to maximize their ability to take up Fe (Maldonado & Price 1996; Mioni et al. 2005; Marchetti et al. 2009). Phytoplankton species display different Fe uptake strategies and have differing biological requirements (Hutchins et al. 1999), and diatoms are particularly sensitive to Fe limitation (Miller et al. 1991; Morel et al. 1991). Larger diatoms have a higher Fe requirement than smaller diatoms, and phytoplankton communities shift to larger sizes (>10 µm) when Fe is added (De Baar et al. 2005). If the concentration of Fe in the Southern Ocean increases over the next century as predicted, the resulting community shifts could have major implications on the efficacy of the biological pump.

Collective uncertainties regarding how Southern Ocean phytoplankton will be impacted by global change highlight the need for experiments that are designed to assess the individual, synergistic, and antagonistic effects of multiple variables (Gunderson et al. 2016). In this study, two different experimental approaches were employed to investigate how predicted future conditions in the Southern Ocean may affect Ross Sea microorganisms, including phytoplankton and bacteria, and the rates at which they take up nitrate (NO$_3^-$) and dissolved inorganic carbon (DIC). The first experiment used a semi-continuous method of culturing to assess the impact of elevated temperature and Fe availability on a Ross Sea microbial assemblage. The second experiment used the same microbial community that had been used in the first experiment, and it occurred directly after the completion of the first experiment. During the second experiment, a continuous method of culturing was used, and high CO$_2$ conditions were tested in addition to
elevated temperature and Fe. Two approaches were used because the semi-continuous approach resembles laboratory batch cultures in the literature, while the continuous ecostat technique mimics natural environmental processes by slowly delivering nutrients over time. The continuous approach also facilitates consistent, quantitative removal of biomass that allows competitive displacement to occur. As a result, those microorganisms that are better suited to the experimental conditions that are being tested will outcompete and have a higher abundance than those less adapted (Hutchins et al. 2003; Pickell et al. 2009).

**MATERIALS AND METHODS**

*Sample Collection and Experimental Setup*

The initial microbial community used for this experiment was collected on January 16, 2013 from the ice edge at McMurdo Sound (77° 36.999’ S, 165° 28.464’ E). Whole seawater was brought to the surface from ~3 meters depth using a trace metal clean diaphragm pump, poured into acid washed cubitainers, and covered with dark plastic bags to reduce light as previously described (Bertrand et al. 2015). Diluent media was prepared by collecting additional seawater that had passed through a 0.2 µm in-line cartridge filter. Half of the diluent media was left unamended, while the remainder was amended with Fe (1 nmol L⁻¹ addition as FeCl₃). A helicopter was used to transport the seawater to the laboratory at McMurdo Station, where the intact microbial community was used to set up the semi-continuous and continuous culture experiment. Initial measurements were completed in the laboratory following collection of the seawater to determine concentrations of chlorophyll a, bacterial abundance, and the concentrations of
total dissolved nitrogen (TDN), NO$_3^-$, nitrite (NO$_2^-$), ammonium (NH$_4^+$), phosphate (PO$_4^{3-}$), silicate (Si), and dissolved organic carbon (DOC). Samples for dissolved nutrients were measured by collecting filtrate that had passed through a GF/F filter (Whatman; nominal pore size 0.7µm; combusted at 450°C for 2 hours) and stored frozen (-20°C) until analysis. Experiments were also set up to measure uptake rates of dissolved inorganic carbon (DIC) and NO$_3^-$ (described in detail below) by the initial microbial community.

*Semi-continuous experiment:* The semi-continuous experiment assessed how elevated temperature and Fe affect nutrient utilization by Ross Sea microorganisms (Table 1). Seawater collected at the ice edge was distributed into 2.7 L PETG bottles that had been acid-washed (trace metal grade HCl) following standard trace metal clean protocols. Half of the bottles received a 1 nmol L$^{-1}$ addition of Fe. Bottles were then placed inside two plexiglass incubators that were set at either 0°C or 4°C. Due to water limitations, it was only possible to prepare one replicate per experimental condition. The two temperature levels were thermostatically controlled using a recirculating heater/chiller system. Incubators were positioned outside and screening was used so that bottles would be exposed to ambient light levels. The semi-continuous experiment lasted for 16 days, and concentrations of chlorophyll $a$ were measured every day during this time. Because biomass of the ice edge community was low, additional sampling did not occur until Day 11 to allow the microorganisms to grow and adjust to the new conditions. Dilutions were performed on Days 11, 14, and 16, by adding diluent media and dividing the communities into duplicate 2.7 L PETG bottles on Day 11, and, further dividing the communities into triplicate bottles on Day 14. Dilutions were performed at ratios ranging
from 1:6 to 1:2, based on a treatment’s average *in vivo* fluorescence. On the days that
dilutions were performed, bacterial abundance and concentrations of dissolved nutrients
including all of the same substrates that were initially analyzed upon collection were
measured both before and after dilution events. On Days 12 and 15, the concentrations of
a smaller set of dissolved nutrients (NO$_3^-$, PO$_4^{3-}$, and Si) were also measured.

*Continuous experiment:* The continuous experiment employed a fully factorial
design to assess the interactive effects of temperature, Fe, and CO$_2$ level on nutrient
utilization by Ross Sea microorganisms (Table 1). The same incubators that were used
for the semi-continuous experiment were used for the continuous experiment, however,
for this experiment the incubators were outfitted to function as continuous culture
systems termed ‘Ecostats’ (Hutchins et al. 2003; Hare et al. 2007; Feng et al. 2009, 2010;
Spackeen et al. 2017). To run the experiment in continuous mode, diluent (either
unamended or amended with Fe) was delivered to the incubation bottles through Teflon
lines that entered the bottles at the top and extended to the bottom. Adjustable peristaltic
pumps were used to control the dilution rate, which was set to 0.4 day$^{-1}$ for all treatments.
This rate was chosen based on average phytoplankton growth rates in the Ross Sea
(Smith et al. 1999). Incubation bottles were bubbled with either 400 ppm or 800 ppm
CO$_2$/air mixtures (Praxair) that had been commercially prepared. In order to re-suspend
the cells that had settled to the bottom, the incubation bottles were secured to a plexiglass
rack that rotated to its side every 5 minutes. This rotating feature enables the biomass
within the ecostat bottles to be homogenously removed through the outflow lines, which
allows growth rates and loss rates of the community to equilibrate (Hutchins et al. 2003).
With the continuous approach, conditions become and remain relatively uniform
throughout the experiment even though subtle changes in community structure and physiology are continuously occurring (Macintyre & Cullen 2005; Pickell et al. 2009).

The continuous experiment lasted for 10 days. Day 0 for this experiment began the same day as the completion of the semi-continuous experiment, following the dilution of the incubation bottles. On Day 2, the incubation bottles started to continuously receive diluent and be bubbled with CO$_2$. Sampling occurred on Day 0 and did not occur again until Day 3 to give the community time to adjust to the new experimental conditions (i.e. continuous diluent and CO$_2$). On Days 0, 3, 6, and 10, samples for bacterial abundance and for a large set of dissolved nutrients (TDN, NO$_3^-$, NO$_2^-$, NH$_4^+$, PO$_4^{3-}$, Si, DOC) were collected. Additionally, every day starting on Day 3, concentrations of chlorophyll $a$, NO$_3^-$, PO$_4^{3-}$, and Si were measured.

Uptake rates of NO$_3^-$ and DIC were measured on Days 0, 4, 6, 8, and 10 (described below). On Day 6, only uptake rates for the 0°C treatments were measured, as this day was the final time point for these treatments. This was necessary due to lack of growth at 0°C, and to ensure that final measurements were taken before the biomass was too low or washed out. Therefore, measurements on Days 8 and 10 only include the 4°C treatments.

**Nutrient Uptake Experiments**

Stable isotope tracer techniques, using $^{15}$N and $^{13}$C labeled substrates, were used to measure uptake rates of NO$_3^-$ and DIC. For the initial uptake experiments that were completed following collection of the seawater, 1 L PETG bottles (filled to capacity ~1.2 L) were used, and during the continuous experiment, polycarbonate conical bottles (230 mL) were used. All bottles used for uptake experiments were acid washed (HCl) and
rinsed with copious amounts of ultrapure water (Barnstead). Uptake experiments were completed by drawing volume from the experimental ecostat bottles using an acid washed syringe. During sampling events, only ~10% of the incubation bottle volume was removed to minimize disruption to the microbial community (Hutchins et al. 2003). NO$_3^-$ and DIC uptake rates were measured by adding $^{15}$N-labeled potassium nitrate (K$^{15}$NO$_3$; 98%) and $^{13}$C-labeled NaH$^{13}$CO$_3$ (99%; Cambridge Isotope Laboratories) to the uptake bottles at an estimated 10% enrichment above background concentrations. Bottles were incubated for ~8 hours under the same light and temperature conditions as the experimental incubation bottles. At the end of the incubation, uptake rates of two different microbial size fractions were determined. First, volume was passed through a 5.0 µm Sterlitech silver membrane filter to collect the larger size fraction of microorganisms (>5.0 µm). Filtrate was then collected and passed through a GF/F filter to collect the smaller size fraction of microorganisms (0.7 – 5.0 µm). Samples were stored at -20°C until the isotopic enrichment of $^{15}$N and $^{13}$C could be assessed on a Europa 20/20 isotope ratio mass spectrometer. Absolute uptake rates were calculated according to Dugdale & Wilkerson (1986) for $^{15}$N-labeled NO$_3$ and Hama et al. (1983) for $^{13}$C-labeled HCO$_3$. The retention of bacteria on GF/F filters was quantified and was 88.8%.

**Particulate and Dissolved Analyses**

Particulate concentrations of nitrogen (PN) and carbon (PC) were analyzed on a Europa 20/20 isotope ratio mass spectrometer at the same time that isotope enrichment was measured. Samples for bacterial abundance were preserved with paraformaldehyde to a final concentration of 0.5%, allowed to set for ~20 minutes, and subsequently flash
frozen using liquid nitrogen and stored at -80°C. Bacterial abundance was quantified by staining the samples with SYBR Green (Invitrogen) and then enumerating them on a high-speed sorter flow cytometer (BD Influx Cell Sorter).

TDN and DOC samples were measured on a Shimadzu 5000A TOC-V/TNM (Detection Limit (DL) 2 \( \mu \)mol N L\(^{-1} \), 5 \( \mu \)mol C L\(^{-1} \); Sharp et al. 2004). Deep-sea water samples were used as references during the TDN/DOC analysis to ensure analytical accuracy (University of Miami consensus reference material program, Hansell 2005). Coulometry (CM5230, UIC) was used to measure DIC (King et al. 2011). Software (CO2SYS) was used to calculate pCO\(_2\) using measurements of pH and DIC (Lewis & Wallace 1998). Concentrations of NO\(_3^-\), NO\(_2^-\), PO\(_4^{3-}\), and Si were measured in duplicate on a Lachat QuickChem 8500 autoanalyzer (DL 0.03 \( \mu \)mol N L\(^{-1} \), DL 0.03 \( \mu \)mol P L\(^{-1} \), DL 0.05 \( \mu \)mol Si L\(^{-1} \); Parsons et al. 1984). Concentrations of NH\(_4^+\) were analyzed in triplicate on a Shimadzu UV-1601 spectrophotometer with the phenol-hypochlorite method (DL 0.05 \( \mu \)mol N L\(^{-1} \); Koroleff 1983).

**Data Analysis and Statistics**

The open source RStudio program (version 0.99.490) was used for all statistical tests. The Shapiro-Wilk test was used to check whether data were normally distributed; data that were not normally distributed were log-transformed prior to further statistical analyses. To determine statistical differences, ANOVAs were performed followed by the post-hoc Tukey’s tests to locate the means of the treatments that were significantly different from one another. Means were considered significantly different if the p value was \( \leq 0.05 \). Statistical tests were only done for the continuous experiment as the semi-continuous experiment lacked the necessary replication.
RESULTS

Semi-continuous Experiment

The initial concentration of chlorophyll $a$ measured at the McMurdo Sound ice edge site was low, less than 1 µg Chl $a$ L$^{-1}$ (Table 2). During the semi-continuous experiment, chlorophyll $a$ concentrations remained near starting concentrations for all treatments for nearly a week (Figure 1). Differences between treatments started to occur on Day 7, when the concentrations of chlorophyll $a$ at 4°C became noticeably higher than concentrations at 0°C (Figure 1). For the remainder of the semi-continuous experiment, the 0°C treatments only increased by ~0.5 to 1 µg Chl $a$ L$^{-1}$ between dilutions, with the 0 +Fe treatment increasing more than the 0 Control treatment (Table 3). At 4°C, however, concentrations of chlorophyll $a$ increased by an order of magnitude. The 4 +Fe treatment increased the most, by ~12 to 14 µg Chl $a$ L$^{-1}$ between dilutions, which was nearly 5 units higher than the change observed in the 4 Control treatment (Table 3).

The pattern in abundance of bacterial cells was opposite the pattern observed for chlorophyll $a$. In the first eleven days of the experiment, bacterial abundance increased at 0°C, and decreased at 4°C (Table 3). By the time the first dilution was performed on Day 11, there were ~9 E+05 cells mL$^{-1}$ more at 0°C than at 4°C. During the rest of the semi-continuous experiment, bacterial abundance at 0°C remained higher than at 4°C, but the difference in abundance between the temperatures became increasingly smaller as bacterial growth increased at 4°C, and slowed down and then declined at 0°C (Table 3).

At the beginning of the experiment, NO$_3^-$ had the highest concentration of the TDN species, >50% of the TDN pool, with an initial concentration of 10 µmol N L$^{-1}$
At a starting concentration of 6.56 µmol N L$^{-1}$, DON also comprised a large portion of the TDN pool. Concentrations of NH$_4^+$ were 1.27 µmol N L$^{-1}$, less than 10% of the TDN pool. Over the course of the semi-continuous experiment, changes in the concentrations of nutrients were generally related to the response of phytoplankton; hence, the magnitude of change between dilution events (i.e., drawdown) was greater at 4°C than at 0°C treatments. This was particularly noticeable for NO$_3^-$ and PO$_4^{3-}$. Prior to the first dilution event on Day 11, the concentration of these two substrates had barely changed in the 0°C treatments. At 4°C, however, NO$_3^-$ and PO$_4^{3-}$ were nearly depleted with lowest concentrations occurring in the 4 +Fe treatment (Table 3). This general pattern also occurred during Days 11-14 and 14-16 for both NO$_3^-$ and PO$_4^{3-}$. Changes in Si and organic nutrients, both DON and DOC, had less consistent patterns than the other nutrients that were analyzed (Table 3).

**Continuous Experiment**

Trends that emerged during the semi-continuous experiment continued once microorganisms were continuously receiving diluent. At all time points, concentrations of chlorophyll $a$ at 0°C remained < 1 µg Chl $a$ L$^{-1}$, and concentrations at 4°C were significantly higher ($p < 0.001$) than at 0°C (Figure 2). At 4°C, concentrations of chlorophyll $a$ increased until Day 3, gradually decreased until Day 7, and then increased until the end of the experiment. Throughout the experiment, treatments with Fe had higher concentrations of chlorophyll $a$ than treatments without Fe at both 0°C and 4°C (Figure 2). Chlorophyll $a$ in both the 0 Control and 0 +CO$_2$ treatments were significantly lower ($p < 0.001$) than the 0 +Fe and 0 Combined treatments on Day 0 (Table 4). On Days 3 and 6, the 0 +CO$_2$ treatment remained significantly lower ($p < 0.02$) than both of
the treatments with Fe, and 0 Control was significantly lower (p < 0.04) than the 0 +Fe treatment on Day 6. For the 4°C treatments, on Days 0 and 6, the 4 Control and 4 +CO₂ treatments were significantly lower (p < 0.04) than the treatments with Fe (Table 4). This trend was not significant on Days 3 and 10 likely due to high variation.

During the continuous experiment, bacterial abundances were similar amongst all treatments and followed a similar pattern over time. Treatments were not significantly different from one another at any time point, with the exception of Day 0, where the 0 Control and 0 +CO₂ treatments had significantly higher (p < 0.02) bacterial abundances than the 4 Control and 4 +CO₂ treatments (Table 4). In all treatments, bacterial abundances increased from Day 0 until Day 6. During the remaining days of the experiment, abundances decreased in all of the 4°C treatments.

Concentrations of dissolved nutrients were similar in pattern to those in the semi-continuous experiment. In general, concentrations of most substrates were higher at 0°C than 4°C (Table 4), reflecting the phytoplankton response (i.e., higher concentrations of chlorophyll a resulted in lower concentrations of nutrients). At 0°C, there were no distinct patterns for most substrates, however, concentrations of both NO₃⁻ and DON were lower in treatments where Fe was added. At 4°C, concentrations of NO₃⁻ and PO₄³⁻ were considerably lower in treatments with added Fe.

At the time of collection absolute NO₃⁻ uptake rates (ρNO₃) of the two size fractions were nearly the same (0.51 ± 0.1 and 0.52 ± 0.1 nmol N L⁻¹ h⁻¹ for smaller and larger microorganisms, respectively). Looking at the treatments, at 0°C, smaller microorganisms had significantly higher (p < 0.04) ρNO₃ than larger microorganisms, while larger microorganisms had significantly higher (p < 0.04) ρNO₃ than smaller
microorganisms at 4°C (Figure 3). This trend occurred at all time points with the exception of the 4 Control treatment on Days 4 and 10, where $\rho_{\text{NO}_3}$ of the two size fractions were not significantly different ($p > 0.05$). For the smaller size fraction, there were no significant differences in $\rho_{\text{NO}_3}$ between treatments except on Day 0, when $\rho_{\text{NO}_3}$ in the 4 +Fe treatment was significantly higher ($p < 0.001$) than the other treatments. In the larger size fraction, $\rho_{\text{NO}_3}$ at 4°C was ~2-3 orders of magnitude higher than at 0°C (Figure 3a and b; $p < 0.001$). At 0°C, there were no significant differences between treatments for larger microorganisms, which is the same trend that was observed for smaller microorganisms. For larger microorganisms at 4°C, the treatments with added Fe had significantly higher ($p < 0.04$) $\rho_{\text{NO}_3}$ than the treatments without Fe on Days 0 and 4. On Days 8 and 10, variability increased and there were no significant differences between treatments.

Showing the same pattern as $\rho_{\text{NO}_3}$, absolute DIC uptake rates ($\rho_{\text{DIC}}$) of the two size fractions were similar initially ($1.0 \pm 0.0 \times 10^{-02}$ and $1.2 \pm 0.2 \times 10^{-02}$ $\mu$mol C L$^{-1}$ h$^{-1}$ for smaller and larger microorganisms, respectively). Only the results for the 4°C treatments are shown because rates were below detection at 0°C (Figure 4). At 4°C, larger microorganisms had significantly higher ($p < 0.02$) $\rho_{\text{DIC}}$ than smaller microorganisms in all treatments and at all time points, with the exception of the 4 Control treatment on Day 10, where $\rho_{\text{DIC}}$ of the two size fractions were not significantly different. For both smaller and larger microorganisms, significant differences between treatments only occurred on Day 0 when the 4 +Fe treatments were higher ($p < 0.001$) than the 4 Control treatments. Although not significant, the treatments with Fe had notably higher $\rho_{\text{DIC}}$ on Days 4, 8, and 10 than the treatments without Fe for larger microorganisms.
DISCUSSION

Temperature Effects

Of all variables tested, temperature caused the largest changes in the microbial community during both the semi-continuous and the continuous experiment. Phytoplankton growth was stimulated by temperature, as evidenced by relatively higher chlorophyll $a$ concentrations and by relatively lower concentrations of most dissolved nutrients at 4°C, indicating nutrient drawdown by the microbial community. The impact of temperature elevation was also reflected in both $\rho_{NO3}$ and $\rho_{DIC}$. Rates by both large and small microorganisms increased, however, the relative increase was greater for large microorganisms in comparison to small. At 0°C, smaller microorganisms were more dominant than large, as shown by the higher concentrations of particulate nitrogen and carbon (data not shown) and by higher $\rho_{NO3}$ in the smaller size fraction (Figure 3). At 4°C, the opposite pattern occurred. Distinct microbial assemblages developed at the two different temperatures, and differences in dominant phytoplankton taxa might explain some of the trends that occurred. In the 0°C treatments, choanoflagellates had the highest relative abundance, while at 4°C *Pseudo-nitzschia* spp, a pennate diatom, dominated the community (Xu et al. in prep). Choanoflagellates are relatively smaller than *Pseudo-nitzschia*, ranging in size from 3-10 µm (King 2005), so it is likely that some choanoflagellates were present in the smaller size fraction and contributing to uptake of $NO_3^-$. Diatoms, including *Pseudo-nitzschia* spp., have evolved strategies to effectively take up $NO_3^-$ (Cochlan et al. 2008), and temperature is known to enhance diatom physiological processes (Reay et al. 2001; Boyd et al. 2016).
Even when conditions were Fe deplete, larger microorganisms had significantly higher rates of nutrient utilization at 4°C than at 0°C. These results were somewhat surprising given that Fe typically limits phytoplankton growth in the Southern Ocean (Martin et al. 1990), and that nutrient limitation is often considered more important than temperature in controlling phytoplankton growth (Marañón et al. 2014). However, other studies show that higher temperatures increase photosynthetic rates of Antarctic phytoplankton (Neori & Holm-Hansen 1982) and that growth rates of some diatom species are enhanced even under Fe deplete conditions (Zhu et al. 2016). Uptake rates of NO$_3^-$ by Southern Ocean phytoplankton have also been shown to increase when incubated at 3°C and 6°C above ambient conditions (Reay et al. 2001). In this same study, Reay et al. (2001) found that specific NO$_3^-$ uptake rates were not impacted when Fe was added at ambient temperatures. They suggested that low in situ temperatures of the Southern Ocean may be as important as direct Fe limitation in regulating NO$_3^-$ use by phytoplankton, and that in certain instances low temperatures play a larger role than Fe in limiting NO$_3^-$ uptake.

During the continuous experiment, $\rho_{\text{DIC}}$ was below detection in the 0°C treatments in both size fractions, and may be reflective of the microbial community that was present. Bacteria were present in the smaller size fraction, and choanoflagellates, the dominant microbial group in the 0°C treatments (Xu et al. in prep), were likely present in both size fractions. Choanoflagellates are heterotrophs and feed primarily on bacteria and particles in the water column (Buck & Garrison 1983; King 2005). Therefore, heterotrophy rather than autotrophy, was the primary carbon acquisition strategy that was being utilized by the microorganisms at 0°C, and carbon sources other than DIC were being used.
Fe Effects

Although temperature had the greatest impact on the microbial community, Fe addition also led to changes in phytoplankton biomass and nutrient utilization. At 0°C, Fe addition resulted in slightly higher concentrations of chlorophyll a and lower concentrations of some dissolved nutrients, however, the effects of Fe addition were more apparent at 4°C. Temperature elevation and Fe addition in combination had a greater impact on phytoplankton physiology than temperature elevation alone. During both the semi-continuous and continuous experiment, concentrations of chlorophyll a were consistently highest in treatments where both temperature was elevated and Fe was added (Figures 1 and 2). On certain days, this was also the case for $\rho_{\text{NO}_3}$ and $\rho_{\text{DIC}}$, and the synergistic effects of temperature and Fe were more evident in the larger size fraction than the smaller (Figure 3a and b; Figure 4a). The additive impact of warming and Fe addition on Southern Ocean microbial physiology has been documented in other studies looking at a mixed assemblage (Rose et al. 2009), certain diatom species (Zhu et al. 2016; Boyd et al. 2016), and several global change parameters of which temperature and Fe were included (Xu et al. 2014). Results from our study support the idea that warming enhances both diffusion-driven and enzymatically-catalyzed cellular reactions, while Fe addition provides a boost to cellular pathways that require Fe (Hutchins & Boyd 2016). It is also possible that the diatoms present in the initial community in our study were able to use their existing cellular Fe pools more efficiently at 4°C, which may explain why there was a strong effect when temperature alone was elevated and why a shift to diatom-dominated assemblages occurred at 4°C. This concept has been previously proposed,
particularly with respect to pennate diatoms (Hutchins & Boyd 2016), which were the dominant taxa of phytoplankton at 4°C in our study (Xu et al. in prep).

**CO₂ Effects**

Elevated concentration of CO₂ did not have a noticeable impact on the microbial community, either individually or in combination with other factors. Within each temperature level, the +CO₂ treatments had the same patterns as the Controls, and the Combined treatments had the same patterns as the +Fe treatments; thus, any significant changes that occurred were likely a function of temperature and/or Fe. There are a few possible explanations for why elevated CO₂ did not have any observed impacts. The microbial community used in this study may have already been accustomed to living in a high CO₂ environment. Compared to temperate regions, the Southern Ocean has relatively more CO₂ due to both cold sea surface temperatures and to upwelling/deep vertical mixing, which brings CO₂-rich water to the surface. As a result of the relatively higher ambient concentrations of CO₂, it is possible that photosynthesis of Southern Ocean phytoplankton is nearly CO₂-saturated from diffusion. This idea has been suggested previously (Gleitz et al. 1996; Raven et al. 2002; Kranz et al. 2015) as well as disputed (Riebesell et al. 1993). Furthermore, for some phytoplankton, such as diatoms, the concentration of CO₂ required to saturate the site of carbon fixation is much lower at colder temperatures (Young et al. 2015b). It is also possible that variability was too high in our experiment to identify any effects that may have been caused by CO₂. Although the parameters that we tested were not affected by high CO₂ conditions, CCM activity may have been affected. Studies find that even when CCM activity is down-regulated under high CO₂ conditions, other physiological processes such as growth rates and
photosynthesis are not necessarily impacted (Boelen et al. 2011; Crawfurd et al. 2011; Young et al. 2015a).

**Summary**

Our study, like many others, demonstrates the importance of considering multiple variables when investigating the potential impacts of climate change. The Southern Ocean is a complex system with several potentially limiting factors that interact with one another at any given time to limit, or enhance, microbial growth and nutrient utilization. In our study, phytoplankton growth and nutrient utilization were likely limited by both the cold temperature of the water and by low concentrations of Fe. Our results also show that temperature has a strong influence on Southern Ocean diatom physiological processes, and that diatom species may be at a competitive advantage in the future depending on how other physical factors that were not tested in this experiment (e.g., light levels, mixed layer depth, etc.) are altered.

Our study was conducted during the latter stages of the annual Ross Sea phytoplankton bloom, so it is important to recognize that our results might be not be applicable to Southern Ocean microbial assemblages that are dominated by taxa, such as *Phaeocystis antarctica*, that bloom earlier in the season. If predictions about temperature elevation in sea surface waters are correct, the biogeochemistry of the Southern Ocean could change considerably within the next century. Our results imply that a warmer Southern Ocean could increase the duration of the bloom season, and that there may be a shift toward larger phytoplankton groups such as diatoms. Our results also indicate that those microorganisms that thrive in low Fe conditions may be at an advantage. If
diatoms become more prevalent, and NO$_3^-$ and DIC utilization increases, as our data indicate, concentrations of dissolved macronutrients in the upper layer of the water column may decrease compared to present day conditions. This will be the case if stratification of the water column intensifies, as predicted by some studies. Prevalence of diatoms and elevated NO$_3^-$ and DIC uptake rates could also be a positive feedback for the biological pump, resulting in increased export to the deep sea; however, the extent of this export will be contingent upon stratification. Although uncertainties remain regarding how nutrient cycling will be altered in the future, the results from this study clearly show the potential for warming alone and warming and Fe, in combination, to increase uptake rates of NO$_3^-$ and DIC. This study represents a valuable stepping stone toward understanding how nutrient cycling in the Southern Ocean will be altered in the future. This study also highlights the importance of how necessary it is to consider temperature, along with other parameters, when designing studies in the this region.

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Table 1) Experimental design for both the semi-continuous experiment and continuous experiment, showing the name used to describe each treatment, the incubation temperature, the addition of Fe that each treatment received, and the pCO$_2$ level.

<table>
<thead>
<tr>
<th>Semi-continuous</th>
<th>Treatment Name</th>
<th>Temp (°C)</th>
<th>Fe addition</th>
<th>pCO$_2$ (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 Control</td>
<td>0</td>
<td>--</td>
<td></td>
<td>400</td>
</tr>
<tr>
<td>0 +Fe</td>
<td>0</td>
<td>1 nmol L$^{-1}$</td>
<td></td>
<td>400</td>
</tr>
<tr>
<td>4 Control</td>
<td>4</td>
<td>--</td>
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<tr>
<td>4 +Fe</td>
<td>4</td>
<td>1 nmol L$^{-1}$</td>
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<table>
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<th>Continuous</th>
<th>Treatment Name</th>
<th>Temp (°C)</th>
<th>Fe addition</th>
<th>pCO$_2$ (ppm)</th>
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<td>0</td>
<td>--</td>
<td></td>
<td>400</td>
</tr>
<tr>
<td>0 +Fe</td>
<td>0</td>
<td>1 nmol L$^{-1}$</td>
<td></td>
<td>400</td>
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<tr>
<td>0 +CO$_2$</td>
<td>0</td>
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<td></td>
<td>800</td>
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<td>0 Combined</td>
<td>0</td>
<td>1 nmol L$^{-1}$</td>
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<tr>
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<td>1 nmol L$^{-1}$</td>
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Table 2) Average concentrations ± 1 standard deviation of chlorophyll a (Chl a), bacterial abundances (BA), and dissolved nutrient concentrations. Measurements are from the McMurdo Sound site water, and from each treatment in the semi-continuous experiment on Day 11 and Day 14 after dilutions had been performed (post dilution). Concentrations of Chl a are reported as µg Chl a L⁻¹, bacterial abundances (BA) are displayed as 10⁵ cells mL⁻¹, and concentrations of dissolved nutrients are in units of µmol of either N, C, P, or Si L⁻¹. On Day 11, the standard deviations are calculated from pseudo replicates. For the Site Water and on Day 14, the standard deviations are calculated from treatment replicates.

<table>
<thead>
<tr>
<th></th>
<th>Chl a</th>
<th>BA</th>
<th>NH₄⁺</th>
<th>NO₃⁻</th>
<th>DON</th>
<th>DOC</th>
<th>PO₄³⁻</th>
<th>Si</th>
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<tbody>
<tr>
<td></td>
<td>µg Chl a L⁻¹</td>
<td>10⁵ cells mL⁻¹</td>
<td>µmol N L⁻¹</td>
<td>µmol N L⁻¹</td>
<td>µmol N L⁻¹</td>
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<td>µmol Si L⁻¹</td>
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<tr>
<td>Site Water (Day 0)</td>
<td>0.39 ± 0.1</td>
<td>9.01 ± 0.5</td>
<td>1.27 ± 0.1</td>
<td>10.0 ± 0.0</td>
<td>6.56 ± 0.4</td>
<td>61.6 ± 9.3</td>
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<td>62.2 ± 1.6</td>
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<td>Day 11 (post dilution)</td>
<td>0 Control</td>
<td>0.29 ± 0.0</td>
<td>11.8</td>
<td>1.55 ± 0.1</td>
<td>9.77 ± 0.0</td>
<td>4.94 ± 0.1</td>
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<td>0.37 ± 0.0</td>
<td>11.7</td>
<td>1.45 ± 0.1</td>
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<td>5.91 ± 0.1</td>
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<td>2.87</td>
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<td>3.67 ± 0.2</td>
<td>2.23</td>
<td>1.34 ± 0.1</td>
<td>7.55 ± 0.0</td>
<td>4.89 ± 0.1</td>
<td>59.0 ± 0.2</td>
<td>0.68 ± 0.0</td>
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<tr>
<td>Day 14 (post dilution)</td>
<td>0 Control</td>
<td>0.15 ± 0.0</td>
<td>9.14 ± 0.6</td>
<td>1.63 ± 0.1</td>
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<td>9.65 ± 0.1</td>
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<tr>
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<td>4 Control</td>
<td>1.67 ± 0.1</td>
<td>1.48 ± 0.2</td>
<td>1.43 ± 0.1</td>
<td>8.32 ± 0.3</td>
<td>5.02 ± 0.5</td>
<td>58.6 ± 0.7</td>
<td>0.77 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>4 +Fe</td>
<td>2.62 ± 0.3</td>
<td>1.16 ± 0.2</td>
<td>1.53 ± 0.1</td>
<td>8.11 ± 0.0</td>
<td>4.89 ± 0.4</td>
<td>58.7 ± 1.6</td>
<td>0.72 ± 0.0</td>
</tr>
</tbody>
</table>
Table 3) Changes (Δ) in concentrations for numerous parameters calculated between dilution events during the semi-continuous experiment. These calculations were calculated by taking the difference between concentrations measured before a dilution event and subtracting them from concentrations measured directly after the previous dilution event had occurred. Concentrations of chlorophyll a (Chl a) are reported as µg Chl a L⁻¹, bacterial abundances (BA) are displayed as 10⁵ cells mL⁻¹, and concentrations of dissolved nutrients are in µmol of either N, C, P, or Si L⁻¹. On Days 14-16, Δ Chl a was not available.

<table>
<thead>
<tr>
<th>Days</th>
<th>Δ Chl a</th>
<th>Δ BA</th>
<th>Δ NH₄⁺</th>
<th>Δ NO₃⁻</th>
<th>Δ DON</th>
<th>Δ DOC</th>
<th>Δ PO₄³⁻</th>
<th>Δ Si</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days 0-11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 Control</td>
<td>0.81</td>
<td>5.28</td>
<td>-0.36</td>
<td>-0.65</td>
<td>-0.59</td>
<td>-2.86</td>
<td>-0.06</td>
<td>3.49</td>
</tr>
<tr>
<td>0 +Fe</td>
<td>1.07</td>
<td>6.83</td>
<td>-0.49</td>
<td>-1.16</td>
<td>4.60</td>
<td>-2.46</td>
<td>-0.08</td>
<td>-1.72</td>
</tr>
<tr>
<td>4 Control</td>
<td>10.2</td>
<td>-0.21</td>
<td>-0.73</td>
<td>-9.84</td>
<td>-1.14</td>
<td>-4.03</td>
<td>-0.82</td>
<td>-11.1</td>
</tr>
<tr>
<td>4 +Fe</td>
<td>14.8</td>
<td>-0.35</td>
<td>-0.89</td>
<td>-9.90</td>
<td>0.03</td>
<td>-3.02</td>
<td>-0.88</td>
<td>-18.1</td>
</tr>
<tr>
<td>Days 11-14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 Control</td>
<td>0.44</td>
<td>1.76</td>
<td>-0.41</td>
<td>-0.11</td>
<td>2.90</td>
<td>-5.47</td>
<td>-0.01</td>
<td>-0.09</td>
</tr>
<tr>
<td>0 +Fe</td>
<td>0.89</td>
<td>1.75</td>
<td>-0.33</td>
<td>-0.32</td>
<td>-0.35</td>
<td>1.83</td>
<td>-0.02</td>
<td>-1.02</td>
</tr>
<tr>
<td>4 Control</td>
<td>7.32</td>
<td>3.98</td>
<td>-0.95</td>
<td>-6.57</td>
<td>0.49</td>
<td>0.06</td>
<td>-0.55</td>
<td>-12.5</td>
</tr>
<tr>
<td>4 +Fe</td>
<td>12.0</td>
<td>4.41</td>
<td>-1.10</td>
<td>-7.51</td>
<td>1.59</td>
<td>5.00</td>
<td>-0.68</td>
<td>-20.2</td>
</tr>
<tr>
<td>Days 14-16</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>0 Control</td>
<td>-4.74</td>
<td>0.02</td>
<td>-0.04</td>
<td>-0.62</td>
<td>-6.82</td>
<td>0.00</td>
<td>-8.54</td>
<td></td>
</tr>
<tr>
<td>0 +Fe</td>
<td>-4.81</td>
<td>-0.04</td>
<td>-0.11</td>
<td>-0.65</td>
<td>-8.38</td>
<td>0.00</td>
<td>4.09</td>
<td></td>
</tr>
<tr>
<td>4 Control</td>
<td>1.38</td>
<td>-0.91</td>
<td>-1.58</td>
<td>-1.00</td>
<td>-8.09</td>
<td>-0.21</td>
<td>-4.40</td>
<td></td>
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<tr>
<td>4 +Fe</td>
<td>1.75</td>
<td>-1.01</td>
<td>-6.11</td>
<td>-0.33</td>
<td>-12.0</td>
<td>-0.50</td>
<td>-2.53</td>
<td></td>
</tr>
</tbody>
</table>
Table 4) Average concentrations ± 1 standard deviation of chlorophyll a (Chl a), bacterial abundances (BA), and dissolved nutrient concentrations on Day 0, Day 3, Day 6, and Day 10 of the continuous experiment. Day 0 of the semi-continuous experiment corresponds to post dilution water from Day 16 of the semi-continuous experiment. Day 6 was the final time point for the 0°C treatments, and Day 10 was the final time point for the 4°C treatments. Concentrations of chlorophyll a (Chl a) are reported as μg Chl a L⁻¹, bacterial abundances (BA) are displayed as 10⁵ cells mL⁻¹, and concentrations of dissolved nutrients are in units of µmol of either N, C, P, or Si L⁻¹. For Chl a and BA, letters next to the value indicate whether or not treatments are significantly different from one another (p ≤ 0.05).

<table>
<thead>
<tr>
<th></th>
<th>Day 0 (post dilution water on day 16)</th>
<th>Day 3</th>
<th>Day 6</th>
<th>Day 10</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chl a µg Chl a L⁻¹</td>
<td>BA 10⁵ cells mL⁻¹</td>
<td>NH₄⁺ µmol N L⁻¹</td>
<td>NO₃⁻ µmol N L⁻¹</td>
</tr>
<tr>
<td>0 Control</td>
<td>0.12 ± 0.0 a</td>
<td>4.72 ± 0.3 b</td>
<td>1.60 ± 0.2</td>
<td>9.87 ± 0.0</td>
</tr>
<tr>
<td>0 +Fe</td>
<td>0.27 ± 0.0 a</td>
<td>4.65 ± 0.2 ab</td>
<td>1.59 ± 0.1</td>
<td>9.78 ± 0.0</td>
</tr>
<tr>
<td>0 +CO₂</td>
<td>0.13 ± 0.0 d</td>
<td>4.72 ± 0.3 b</td>
<td>1.60 ± 0.2</td>
<td>9.87 ± 0.0</td>
</tr>
<tr>
<td>0 Combined</td>
<td>0.28 ± 0.0 a</td>
<td>4.65 ± 0.2 ab</td>
<td>1.59 ± 0.1</td>
<td>9.78 ± 0.0</td>
</tr>
<tr>
<td>4 Control</td>
<td>3.09 ± 0.4 b</td>
<td>4.15 ± 0.1 a</td>
<td>1.20 ± 0.2</td>
<td>8.48 ± 0.3</td>
</tr>
<tr>
<td>4 +Fe</td>
<td>5.79 ± 0.7 a</td>
<td>4.40 ± 0.1 ab</td>
<td>1.09 ± 0.1</td>
<td>6.31 ± 0.2</td>
</tr>
<tr>
<td>4 +CO₂</td>
<td>3.09 ± 0.4 b</td>
<td>4.15 ± 0.1 a</td>
<td>1.20 ± 0.2</td>
<td>8.48 ± 0.3</td>
</tr>
<tr>
<td>4 Combined</td>
<td>5.79 ± 0.7 a</td>
<td>4.40 ± 0.1 ab</td>
<td>1.09 ± 0.1</td>
<td>6.31 ± 0.2</td>
</tr>
</tbody>
</table>
**Figure 1** Chlorophyll $a$ concentrations of the whole community (>0.07 µm) over the course of the semi-continuous experiment. Dilution events that occurred on Day 11 and Day 14 are indicated by the dotted line.
Figure 2) Mean chlorophyll $a$ concentrations ± 1 standard deviation of the whole community (>0.07 µm) over the course of the continuous experiment. Day 6 was the final time point for the 0°C treatments, because it was no longer growing.
Figure 3) Mean absolute NO$_3^-$ uptake rates ± 1 standard deviation measured on Day 0 (A), Day 4 (B), Day 6 (C), Day 8 (D), and Day 10 (E) during the continuous experiment. Treatments marked as NM on Day 0 were not measured because CO$_2$ levels had not been manipulated at that point. The smaller plots within the Day 0 and Day 4 figures are the 0°C treatments plotted on a smaller scale. Only the 0°C treatments were measured on Day 6, the final time point for the 0°C treatments. Note that the scale is larger for the 4°C treatments on Day 8 and Day 10, compared to Day 0 and Day 4. Letters above each bar indicate whether or not treatments are significantly different from each other. Within each plot, bars that do not have a letter in common are significantly different from one another (p ≤ 0.05).
Figure 4) Mean absolute DIC uptake rates ± 1 standard deviation measured on Day 0 (A), Day 4 (B), Day 8 (C), and Day 10 (D) during the continuous experiment. Treatments marked as NM on Day 0 were not measured because CO₂ levels had not been manipulated at that point. Note that the scale is larger on Day 10 compared to the other plots. Letters above each bar indicate whether or not treatments are significantly different from each other. Within each plot, bars that do not have a letter in common are significantly different from one another (p ≤ 0.05).
CHAPTER 4

STOICHIOMETRIC N:P RATIOS, TEMPERATURE, AND IRON IMPACT CARBON AND NITROGEN UPTAKE BY ROSS SEA MICROBIAL COMMUNITIES

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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 experiments were conducted to study how these predicted changes will impact nutrient utilization and community structure. Natural microbial communities from Terra Nova Bay and McMurdo Sound from the Ross Sea, Antarctica were used. The Terra Nova Bay community was amended with Fe, and grown under a range of N:P ratios. The McMurdo Sound community was incubated under select N:P ratios, with and without elevated temperatures and added Fe. Absolute uptake rates of bicarbonate, nitrate, and amino acids by two size fractions (0.7-5.0 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. Temperature was also identified as the principal driver to influence phytoplankton community composition. Results indicate that changing the dissolved N:P ratio can potentially alter nutrient uptake rates, however, the impact of temperature and Fe are greater.
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 and Weber, 2012], and it has been widely used as a fundamental principle to shape our understanding of marine ecology [Arrigo, 2005; Gruber and Deutsch, 2014]. There are a number of processes that influence stoichiometric ratios in the marine environment, however, and research from the past few decades has concluded that the Redfield ratio is often the exception rather than the rule [Heckey et al., 1993; Geider and 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 [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 and Weber, 2012; Planavsky, 2014], while haptophytes, such as *Phaeocystis antarctica*, have higher N:P and C: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 conditions [Rhee, 1973] and growth phase [Arrigo, 2005]. The cellular components that are most active within a phytoplankton cell depend on whether the cells are in exponential growth phase or growing more slowly due to limited resources as they approach stationary phase. Some cellular components that are used to acquire resources, such as protein and chlorophyll, have high N:P ratios. In contrast, ribosomal RNA, which is used for growth has relatively low N:P ratios [Falkowski, 2000; Geider and 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; Tatters et al., in prep], 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 and 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 [Deutsch and Weber, 2012]. 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 and 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 [De La Rocha et al., 2000; Dong et al., 2000; Berman-Frank et al., 2001], while others find the opposite trend [Doucette and Harrison, 1991; Maldonado and 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 [IPCC, 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 [Sedwick and DiTullio, 1997; Raiswell et al., 2008; Hutchins and Boyd, 2016]. Because
the majority of dissolved Fe is complexed with organic ligands, the accessibility of the Fe to different phytoplankton groups will depend on the chemical structure of the ligand [Geldhill and Buck, 2012, Hutchins et al. 1999].

Similarities between dissolved nutrient ratios and phytoplankton stoichiometry suggest they are closely coupled [Arrigo, 2005]. Small changes in nutrient stoichiometry have the potential to significantly affect primary production [Karl et al., 1995]. Furthermore, changes in nutrient ratios can affect the quality of food at the base of the food web, which has the potential to impact organisms at higher trophic levels [Sterner and 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 and 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 global change will influence nutrient stoichiometry and the utilization of nutrients by phytoplankton. In this study, two experiments were conducted within the Ross Sea to determine how changing the dissolved N:P ratio of the seawater impacts nutrient uptake and phytoplankton community composition. The first experiment assessed the phytoplankton response to a wide range of dissolved N:P ratios. The second experiment 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.
MATERIALS AND METHODS

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 January 22, 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 December 29, 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 ~3-5 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.

Bioassays

TNB Experiment: Seawater was evenly distributed into 6 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. To relieve potential Fe stress and ensure that we would see a response, all treatments, with the exception of the Control, also received a 1 nmol L$^{-1}$ addition 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 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 *). After nutrients were added
to the cubitainer, seawater was gently mixed and distributed into 2 L 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 $0^\circ$C that received $\sim45 \mu$mol photons m$^{-2}$ s$^{-1}$ of constant light. This light level was selected because it is a level commonly observed in the open water and under the sea ice. The community used in our experiment was collected from the ice edge; thus, the microorganisms would have recently experienced both open water and under ice light regimes.

The experiment lasted 10 days. Chlorophyll $a$, NO$_3^-$ and PO$_4^{3-}$ concentrations were measured several times during the experiment (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 DIC were measured (described in detail below); on Day 0 and Day 10 uptake rates of amino acids were also measured. (Limits on available water precluded measuring all parameters at all time points for both the TNB and MMS experiments.)

**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 ($0^\circ$C and $4^\circ$C), decreased N:P ratios ($\sim7.4$), increased
N:P ratios (~22), and Fe addition (+2 nmol L\(^{-1}\)) (Table 1). Bottles were incubated for 7 days in two 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 during the austral summer when light levels are typically higher. 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. On Day 3 and 5, smaller sampling events took place to measure chlorophyll \(a\) concentrations, NO\(_3^+\), and PO\(_4^+\).

**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 experiments were done in acid washed 500 mL PETG bottles filled to capacity (~600 mLs) 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 h. 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.

**Dissolved Nutrient Analyses**

To measure dissolved nutrient concentrations, seawater was filtered through a
Whatman GF/F filter (combusted at 450°C for 2 h) 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 and Harrison, 1987] respectively. A Shimadzu RF-1501 spectrofluorometer was
used to measure concentrations of dissolved primary amines (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 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 dissolved inorganic carbon (DIC) using the method described in King et al. [2011].

**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 h at 550°C, extracted overnight using hydrochloric acid (1N), and concentrations of orthophosphate were analyzed colorimetrically [Aspila and 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 non-acidified 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, and enumerated microscopically using an Accu-Scope 3032 [Utermöhl 1931]. Species were identified according to Scott & 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 observed. Taxa include: Diatoms (*Pseudo-nitzschia* spp., *Fragilariopsis* spp., other pennate diatoms, centric diatoms), Dinoflagellates, *Phaeocystis* (single cells and colonies), and Other (all other taxa, mostly rare species). We note that the number of cells comprising *Phaeocystis* colonies were determined; hence, the number of individual colonial cells were included in the relative abundance calculation.

**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. One-way ANOVAs 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 ≤ 0.05, means were considered significantly different from one another. To visually assess similarity among phytoplankton communities in the MMS experiment, final abundances were square-root transformed, and Bray Curtis similarities were computed and used to construct a multi-dimensional scale (MDS) plot.

**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 (Table 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.

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$^{-1}$ (Figure 1). Growth was slow for the first 3 days of the experiment, but increased thereafter and remained in exponential growth until the experiment ended. Treatment chlorophyll $a$ concentrations were clustered into two groups, which became evident on Day 9 and 10. 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 Day 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). Similarities in chlorophyll $a$ concentrations between the Control and the N:P extremes are not consistent with most studies from the Southern Ocean that show that phytoplankton growth is stimulated with Fe addition [De Baar et al., 1990; Martin et al., 1990; Boyd et al., 2000; Coale et al., 2004], and may indicate that phytoplankton growth is negatively impacted when dissolved N:P ratios are outside of an optimal stoichiometric range, even when Fe is present. It is also possible that Fe was not limiting to the initial community or that Fe
contamination occurred during the experimental set up. While these scenarios are possible, the 11* treatment generally had the lowest nutrient concentrations compared to the other treatments, and concentrations of particulate carbon and particulate nitrogen were higher in the 11* treatment compared to the Control on Day 10. These results indicate that Fe limitation was likely.

Bacterial abundances increased in all treatments by Day 3 (Figure 2A). Bacterial abundance in the Control was similar to or higher than (p < 0.03) all of the other treatments. These results support studies from Fe-limited HNLC regions that show that Fe addition does not lead to more bacterial growth [Kirchman et al., 2000; Agawin et al., 2006]. By Day 10, bacterial abundance in the Control had decreased relative to Day 3, and all treatments had higher (p < 0.001) abundances than the Control (Figure 2B). Highest bacterial abundances occurred in the same treatments that had the highest concentrations of chlorophyll a by Day 10 (r^2 = 0.49), indicating that the bacterial response is likely coupled to the response of phytoplankton and the availability of organic matter. Bacterial growth in the Southern Ocean is enhanced when labile organic matter is available [Church et al., 2000].

3.1.2 TNB Uptake

DIC: Absolute DIC uptake rates (ρDIC) by the >5.0 μm size fraction were significantly greater (p < 0.001) than the 0.7-5.0 μm size fraction for both the initial community and on Day 3 for all treatments (Figure 3A). By Day 10 the opposite pattern was observed, and the 0.7-5.0 μm size fraction had higher ρDIC than the >5.0 μm size fraction (Figure 3B). Within the treatments, Fe addition alone (11*) and a moderate increase (13*) to the N:P ratio resulted in higher ρDIC compared to the Control for both
size fractions (p < 0.01; Figure 3A and B). Additionally, on Day 3, all treatments with elevated N:P ratios, including 33* and 49*, had higher (p < 0.01) \( \rho_{\text{DIC}} \) than the Control for the >5.0 \( \mu \)m size fraction. For the 0.7-5.0 \( \mu \)m 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 (\( \rho_{\text{AA-C}} \)) of the 0.7-5.0 \( \mu \)m size fraction were significantly greater (p < 0.001) than the >5.0 \( \mu \)m size fraction with the exception of the Control (Figure 3C). In the 0.7-5.0 \( \mu \)m size fraction, the 11* treatment and treatments with elevated N:P ratios had higher (p < 0.01) \( \rho_{\text{AA-C}} \) than the Control. However, none of these treatments were significantly different from each other.

**NO\(_3\)^-:** Within the initial community, absolute NO\(_3\)^- uptake rates (\( \rho_{\text{NO3}} \)) of the 0.7-5.0 \( \mu \)m size fraction were significantly higher (p < 0.03) than the >5.0 \( \mu \)m size fraction (Figure 4A). This pattern continued on Day 3, but was only significant (p < 0.001) in the Control. By Day 10, \( \rho_{\text{NO3}} \) was not significantly different between the size fractions. For both size fractions when the N:P ratio was at 33 and 49, \( \rho_{\text{NO3}} \) was significantly higher (p < 0.02) than the other treatments (Figure 4A). This pattern only occurred on Day 3, and by Day 10, \( \rho_{\text{NO3}} \) of all treatments in both size fractions were similar (Figure 4B). Additionally, within the >5.0 \( \mu \)m size fraction, the 11* and 13* treatments were higher (p < 0.04) than the Control on Day 3 (Figure 4A).

**AA-N:** Absolute AA-N uptake rates (\( \rho_{\text{AA-N}} \)) had the same pattern as \( \rho_{\text{AA-C}} \). For the initial community, \( \rho_{\text{AA-N}} \) of the 0.7-5.0 \( \mu \)m size fraction was more than triple that of the >5.0 \( \mu \)m size fraction (Figure 4C). Significantly higher \( \rho_{\text{AA-N}} \) in the 0.7-5.0 \( \mu \)m size fraction also occurred during the experiment (p < 0.001), with the exception of the
Control. Comparing the treatments, in the 0.7-5.0 µm size fraction, the 33* treatment had higher (p < 0.04) $\rho_{AA-N}$ than the Control, and in the > 5.0 µm size fraction, the 2* treatment was lower (p < 0.01) than the Control.

Although uptake trends varied between substrates as well as over the course of the experiment, several of the findings from this study stand out. First, when the N:P ratio was decreased to 2, $\rho_{DIC}$ and $\rho_{NO_3}$ were not significantly different from the Control in either size fraction at any point during the experiment, indicating that 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 the added substrate, 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 for certain microbial communities, low N:P ratios prevent growth and DIC and NO$_3^-$ utilization from increasing even when Fe stress is eliminated.

Second, our data 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 $\rho_{NO_3}$ significantly increased, the change occurred at the beginning of the experiment (Figure 4A). Fe addition may have been the main factor influencing change on Day 3, however, $\rho_{NO_3}$ 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 [Rhee, 1978; Lomas and Glibert, 2000], and may have occurred during the initial stages of the experiment (Figure 4A) and decelerated by the end of the experiment (Figure 4B). This could explain why there were no statistical differences in $\rho_{\text{NO3}}$ between treatments on Day 10 (Figure 4B).

Finally, the high $\rho_{\text{AA-C}}$ and $\rho_{\text{AA-N}}$ 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 3C and Figure 4C). The considerable amount of uptake by the smaller size fraction is likely attributed to bacteria, as the majority of bacteria (88.8%) at our site were retained on the GFF filter. Higher $\rho_{\text{AA-C}}$ and $\rho_{\text{AA-N}}$ by smaller microorganisms compared to larger microorganisms is the same trend that was observed for $\rho_{\text{DIC}}$ on Day 10, and makes sense given that bacterial abundance increased at the end of the experiment (Figure 2B). Changes to nutrient ratios may have also influenced amino acid uptake rates, yet far less noticeably than Fe addition. In the smaller size fraction, statistical differences were observed for $\rho_{\text{AA-C}}$ when only Fe was added or when N:P ratios were increased, and for $\rho_{\text{AA-N}}$ statistical differences occurred when the N:P ratio was at 13 and 33. When the ratio was decreased to 2, $\rho_{\text{AA-C}}$ and $\rho_{\text{AA-N}}$ were similar to the control, and in the larger size fraction $\rho_{\text{AA-N}}$ was actually 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 [Suttle et al., 1991; Jørgensen et al., 1993; Keil and Kirchman, 1999; Zubkov et al., 2008], and the small amount of
research done in the Southern Ocean show that turnover of amino acids is high [Simon and Rosenstock, 2007] and that utilization is influenced by irradiance [Rivkin and Putt, 1987]. Our data indicate that more research is needed in HNLC regions to examine the affects of Fe addition on dissolved organic nutrient utilization, because of its potential for shaping biogeochemical cycles, particularly with respect to phytoplankton-bacterial interactions.

3.1.3 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). By Day 10, however, particulate C:N ratios were similar to the starting ratio, and C:P ratios were lower than the starting ratio. 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. 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). Because larger cellular stoichiometric changes occurred on Day 3 in comparison to Day 10, this further supports the idea posed earlier 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.
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). Relatively higher concentrations of oxidized nutrients and lower concentrations of reduced nutrients at the MMS site compared to the TNB site 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 (Table 1 and 2). During the MMS experiment, concentrations of Si did not change. This is likely because diatoms were not a major component of the microbial assemblage (Table 4).

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$^{-1}$ (Figure 5). 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 5A). The treatments at 4°C were separated into two groups based on whether Fe was present (Figure 5B). Treatments with Fe had the highest chlorophyll $a$ concentrations, and treatments without Fe had similar concentrations to the 0°C treatments. The lack of response to Fe addition at 0°C indicates that temperature elevation may be necessary to drive an Fe addition response by certain Ross Sea microbial communities.
Temperature elevation, rather than Fe addition or stoichiometric change, resulted in the highest abundances of bacteria cells (Figure 6). Each treatment at 4°C had higher (p < 0.001) bacterial abundances than the respective 0°C treatment that had the same stoichiometric and Fe conditions, except when the N:P ratio was low and Fe was present. The positive relationship between temperature and bacterial growth was expected given that microbial metabolism generally increases as a function of temperature, and it is considered to be among the most important parameters controlling microbial activity [Delille, 2004; Hutchins and Fu, 2017].

3.2.2 MMS Uptake

*DIC:* The initial MMS community had the same pattern as the TNB community in that the >5 µm size fraction had higher $\rho_{\text{DIC}}$ (p < 0.001) than the 0.7-5.0 µm size fraction (Figure 7A). This pattern also occurred during the experiment (p < 0.001), with the exception of the 4°C-Fe set of treatments where $\rho_{\text{DIC}}$ of the two size fractions were not significantly different (Figure 7A). Comparing the treatments to the Control, $\rho_{\text{DIC}}$ significantly increased (p < 0.001) for the >5.0 µm size fraction when Fe was added, and $\rho_{\text{DIC}}$ significantly increased (p < 0.01) for the 0.7-5.0 µm size fraction at 4°C. In the 0.7-5.0 µm size fraction, $\rho_{\text{DIC}}$ also significantly increased (p < 0.01) in the 0°C ambient* treatment.

*AA-C*: For the initial community, $\rho_{\text{AA-C}}$ of the 0.7-5.0 µm size fraction was significantly greater (p < 0.001) than the >5.0 µm size fraction (Figure 7B). Highest $\rho_{\text{AA-C}}$ for both size fractions occurred in the 4°C+Fe set of treatments (Figure 7B). In the >5.0 µm size fraction, Low* and High* N:P ratios at 4°C resulted in higher (p < 0.001) $\rho_{\text{AA-C}}$ than the Control and the 0°C Low and 0°C ambient* treatments.
$NO_3^-$: The $>5.0 \mu m$ size fraction had higher $\rho_{NO3}$ than the 0.7-5.0 $\mu m$ size fraction for the 0°C-Fe, 0°C+Fe, and 4°C+Fe set of treatments (Figure 8A). The opposite pattern occurred in the 4°C-Fe set of treatments (Figure 8A). Taking a closer look at the individual size fractions, $\rho_{NO3}$ in the $>5.0 \mu m$ 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 0.7-5.0 $\mu m$ 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 $\rho_{AA-C}$, highest $\rho_{AA-N}$ also occurred in the 4°C+Fe set of treatments (Figure 8B). In the $>5.0 \mu m$ 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 0.7-5.0 $\mu m$ size fraction, the 4°C+Fe set of treatments, with the exception of the 4°C Ambient* treatment, had higher ($p < 0.02$) $\rho_{AA-N}$ than the 0°C-Fe set of treatments.

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 7A and 8A) 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 and 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 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 $\rho_{\text{DIC}}$ and $\rho_{\text{NO}_3}$ were higher than the Control. This could be due to bacterial uptake of $\text{NO}_3^-$. Bacterial abundance was higher in these treatments than the Control (Figure 2B). Temperature is known to enhance microbial metabolism [Pomeroy and Wiebe, 2001; Delille, 2004], and research has demonstrated that elevated temperature may be equally as important as Fe limitation on impacting nutrient uptake by Southern Ocean microorganisms [Reay et al., 2001; Hutchins and Boyd, 2016]. Finally, in comparison to the larger size fraction, the smaller size fraction was likely able to more efficiently access any available Fe 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 available. These findings may be unique to Phaeocystis-dominated communities, which was the case for this study (discussed below). Phaeocystis growth can decline at higher temperatures [Xu et al., 2014] and other prominent Southern Ocean microorganisms may be at a competitive advantage in a warmer Southern Ocean [Boyd et al., 2016; Petrou et al., 2016; Zhu et al., 2016; Coello-Camba and Agusti, 2017].

In the MMS experiment, $\rho_{\text{AA-C}}$ and $\rho_{\text{AA-N}}$ 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 (Figures 2 and 6). Community differences may also explain why trends between the two experiments differed. For the TNB experiment, Fe addition clearly impacted amino acid utilization by smaller microorganisms (Figure 3C and 4C), while in the MMS experiment increasing Fe and temperature in combination were needed to produce the highest $\rho_{\text{AA-C}}$ and $\rho_{\text{AA-N}}$. This was evident, significantly in some cases, for
both small and large microorganisms (Figure 7B and 8B). 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 is a topic that should be further investigated.

Changes to the dissolved N:P ratio may have also impacted rates, however, in this study the effects 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, $\rho_{NO_3}$ 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. It is possible that for the MMS experiment any impact on $NO_3^-$ uptake rates due to increasing the N:P ratio 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.
3.2.3 MMS Cellular Stoichiometry

By Day 7 of the experiment, cellular particulate ratios were mostly unaffected, with the exception of the treatments that were at 0°C and Fe replete, which had significantly lower particulate C:N ratios 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 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 particulate N:P and C:P ratios [Martiny et al., 2013; Moore et al., 2013].

3.2.4 MMS Phytoplankton Community Composition

The dominant member of the 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 mL⁻¹. There were generally more cells colony⁻¹ at 0°C than at 4°C, yet there were more colonies mL⁻¹ in the 4°C treatments than the 0°C treatments. Abundances of colonies mL⁻¹ were highest in the 4°C+Fe set of treatments (Table 4). Although colonial *Phaeocystis* was dominant, a non-parametric MDS plot allowing for visualization of similarity among the final phytoplankton communities revealed that treatments set at the same temperature clustered together, with the 4°C
Ambient treatment (i.e., the treatment with only temperature manipulated) being in close proximity to the Control (Figure 9). The 0°C treatments were loosely clustered according to N:P supply ratios, and the Fe replete low N:P treatment diverged the most from the others. The 4°C treatments were clustered based on Fe addition. The high N:P treatments clustered both within and among the temperature levels.

The relative abundance of dominant phytoplankton groups other than colonial *Phaeocystis* shows differences in the distribution of groups/species among treatments (Figure 10). Single *Phaeocystis* cells and dinoflagellates were relatively abundant in all treatments. In the 0°C-Fe, 0°C+Fe, and 4°C–Fe set of treatments, dinoflagellates were more abundant than *Phaeocystis* single 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 *Phaeocystis* single cells were relatively more abundant than dinoflagellates.

Diatoms, including centrics, *Fragilariopsis* spp., *Pseudo-nitzschia* spp., and other pennates, comprised a small portion of the overall community. Centric diatoms accounted for less than 1% of the whole community in all treatments, with the 0°C High treatment having the highest relative abundance. *Fragilariopsis* spp. was relatively abundant in the 0°C Low* treatment, comprising 2.5% of the whole community, which was 2-fold more than its abundance in any of the other treatments. For *Pseudo-nitzschia* spp., relative abundance was highest when the temperature was at 4°C and when the N:P ratio was high, regardless of whether Fe was replete or deplete. In all treatments, other pennate diatom species 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 [Hare et al., 2007; Feng et al., 2009; Tatters et al., in prep], Fe addition [Feng et al., 2010], and nutrient ratios [Lagus et al., 2004; De Senerpont Domis et al., 2014; Czerny et al., 2016] 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* was dominant in the initial community [Xu et al., in prep] and usually reaches maximum abundance in the Ross Sea in mid- to late-December [Smith et al., 2000], the time frame when water was collected for the experiment. Temperature elevation, particularly when combined with Fe addition, increased the number of *Phaeocystis* colonies. Increases in colony abundance as a function of both 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* were highest in these treatments (Figure 5).

Although *Phaeocystis* colonies were dominant, there were subtle differences in community composition among treatments, and temperature was the main variable structuring the communities (Figures 9 and 10). The 4°C +Fe set of treatments, in particular, were distinctly different from the treatments at 0°C, and these treatments had lower relative abundances of 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. Another result was the prominence of Fragilariopsis spp. in the 0°C Low* treatment (Figure 10), which might explain why this treatment diverged from the rest in the MDS plot (Figure 9). 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]. Furthermore, P utilization by Fragilariopsis cylindrus was higher than other Southern Ocean phytoplankton species when incubated in culture under Fe-replete conditions [Spackeen et al., in prep]. Perhaps Fe-replete conditions coupled with proportionately higher concentrations of PO$_4^{3-}$ are favorable for Fragilariopsis spp.

Another result was that Pseudo-nitzschia became relatively more dominant at 4°C when the N:P supply ratio was high. 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.

In the MMS study, Phaeocystis dominance 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 semi-stable state. The average C:N:P ratio of phytoplankton influence 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 large 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. In the two studies that we conducted, we present evidence that different dissolved N:P ratios have the potential to impact growth and nutrient utilization by microorganisms. We also found that different dissolved N:P ratios resulted in subtle shifts to the phytoplankton community composition. 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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LITERATURE CITED


Spackeen, J.L., K. Xu, R.E. Sipler, Z. Zhu, D.A. Hutchins, and D.A. Bronk (in prep), Uptake of carbon, nitrogen, and phosphorus under a matrix of temperature and


Tatters, A.O., A. Schnetzer, K. Xu, N.G. Walworth, F-X. Fu, J.L. Spackeen, R.E. Sipler, E.M. Bertrand, J.B. McQuaid, A.E. Allen, D.A. Bronk, K. Gao, J. Sun, D.A. Caron, and D.A. Hutchins (in prep), Interactive effects of temperature, CO₂, and nitrogen source on a coastal California plankton assemblage: I. Diatom community composition and domoic acid production,


Xu, K., et al (in prep)

Zhu, Z., K. Xu, F-X. Fu, J.L. Spackeen, D.A. Bronk, and D.A. Hutchins (2016), A comparative study of iron and temperature interactive effects on diatoms and

Table 1) Experimental design of the TNB experiment and the MMS experiment, showing the name used to describe each treatment, the nutrient ammendments (+NO$_3^-$, +PO$_4^{3-}$, +Fe) made to each treatment, the incubation temperature, and the intial NO$_3^-$ and PO$_4^{3-}$ concentrations and NO$_3^-$:PO$_4^{3-}$ ratio. Note that the * indicates added Fe. All treatments were prepared in triplicate, except for 11 (Control), which was prepared in duplicate due to water limitations, and the High treatment, where a replicate bottle was lost. The NO$_3^-$ and PO$_4^{3-}$ concentrations and the NO$_3^-$:PO$_4^{3-}$ ratio listed for the High treatment came from Day 3 of the experiment because nutrient samples for this treatment were inadvertently collected prior to nutrient ammendments.

<table>
<thead>
<tr>
<th>Treatment Name</th>
<th>+ PO$_4^{3-}$</th>
<th>+ NO$_3^-$</th>
<th>+ Fe</th>
<th>Temp (°C)</th>
<th>NO$_3^-$ µmol N L$^{-1}$</th>
<th>PO$_4^{3-}$ µmol P L$^{-1}$</th>
<th>NO$_3^-$:PO$_4^{3-}$</th>
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</thead>
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<td><strong>TNB Experiment</strong></td>
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<tr>
<td>2*</td>
<td>Yes</td>
<td>--</td>
<td>Yes</td>
<td>0</td>
<td>6.91 ± 0.0</td>
<td>3.29 ± 0.0</td>
<td>2.1 ± 0.0</td>
</tr>
<tr>
<td>11 (Control)</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>0</td>
<td>6.71 ± 0.4</td>
<td>0.59 ± 0.0</td>
<td>11.3 ± 0.7</td>
</tr>
<tr>
<td>11*</td>
<td>--</td>
<td>--</td>
<td>Yes</td>
<td>0</td>
<td>6.71 ± 0.4</td>
<td>0.59 ± 0.0</td>
<td>11.3 ± 0.7</td>
</tr>
<tr>
<td>13*</td>
<td>--</td>
<td>Yes</td>
<td>Yes</td>
<td>0</td>
<td>7.68 ± 0.0</td>
<td>0.61 ± 0.0</td>
<td>12.6 ± 0.0</td>
</tr>
<tr>
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<td>--</td>
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<td>Yes</td>
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<td>16.9 ± 0.0</td>
<td>0.51 ± 0.0</td>
<td>33.3 ± 0.0</td>
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<td>25.0 ± 0.0</td>
<td>0.51 ± 0.0</td>
<td>49.0 ± 0.1</td>
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<td><strong>MMS Experiment</strong></td>
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<tr>
<td>Low</td>
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<td>--</td>
<td>--</td>
<td>0</td>
<td>19.4 ± 0.7</td>
<td>2.66 ± 0.1</td>
<td>7.3 ± 0.2</td>
</tr>
<tr>
<td>Ambient (Control)</td>
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<td>--</td>
<td>--</td>
<td>0</td>
<td>18.7 ± 0.8</td>
<td>1.47 ± 0.1</td>
<td>12.8 ± 0.7</td>
</tr>
<tr>
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<td>--</td>
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<td>35.9 ± 8.5</td>
<td>1.28 ± 0.1</td>
<td>27.9 ± 5.0</td>
</tr>
<tr>
<td>Low*</td>
<td>Yes</td>
<td>--</td>
<td>Yes</td>
<td>0</td>
<td>19.7 ± 0.7</td>
<td>2.63 ± 0.2</td>
<td>7.5 ± 0.3</td>
</tr>
<tr>
<td>Ambient*</td>
<td>--</td>
<td>--</td>
<td>Yes</td>
<td>0</td>
<td>19.6 ± 0.6</td>
<td>1.41 ± 0.1</td>
<td>13.9 ± 0.4</td>
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<tr>
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<td>Yes</td>
<td>0</td>
<td>34.6 ± 0.5</td>
<td>1.57 ± 0.1</td>
<td>22.1 ± 1.0</td>
</tr>
<tr>
<td>Low</td>
<td>Yes</td>
<td>--</td>
<td>--</td>
<td>4</td>
<td>19.3 ± 0.6</td>
<td>2.63 ± 0.1</td>
<td>7.4 ± 0.4</td>
</tr>
<tr>
<td>Ambient</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>4</td>
<td>19.1 ± 0.8</td>
<td>1.54 ± 0.1</td>
<td>12.4 ± 0.0</td>
</tr>
<tr>
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<td>Yes</td>
<td>--</td>
<td>4</td>
<td>33.5 ± 0.8</td>
<td>1.52 ± 0.0</td>
<td>22.1 ± 0.5</td>
</tr>
<tr>
<td>Low*</td>
<td>Yes</td>
<td>--</td>
<td>Yes</td>
<td>4</td>
<td>19.4 ± 0.7</td>
<td>2.63 ± 0.1</td>
<td>7.4 ± 0.3</td>
</tr>
<tr>
<td>Ambient*</td>
<td>--</td>
<td>--</td>
<td>Yes</td>
<td>4</td>
<td>19.3 ± 0.7</td>
<td>1.54 ± 0.1</td>
<td>12.5 ± 0.1</td>
</tr>
<tr>
<td>High*</td>
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<td>Yes</td>
<td>Yes</td>
<td>4</td>
<td>34.4 ± 1.4</td>
<td>1.53 ± 0.1</td>
<td>22.6 ± 0.3</td>
</tr>
</tbody>
</table>
Table 2) 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.

<table>
<thead>
<tr>
<th></th>
<th>DOC (\mu\text{mol C L}^{-1})</th>
<th>NH(_4^+) (\mu\text{mol N L}^{-1})</th>
<th>NO(_2^-) (\mu\text{mol N L}^{-1})</th>
<th>NO(_3^-) (\mu\text{mol N L}^{-1})</th>
<th>DON (\mu\text{mol N L}^{-1})</th>
<th>Urea (\mu\text{mol N L}^{-1})</th>
<th>DPA (\mu\text{mol P L}^{-1})</th>
<th>PO(_4^{3-}) (\mu\text{mol Si L}^{-1})</th>
<th>Si (\mu\text{mol L}^{-1})</th>
<th>NO(_3^- : PO_4^{3-})</th>
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</thead>
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<tr>
<td>TNB Site Water</td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>(Day 10)</td>
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</tr>
<tr>
<td>2*</td>
<td>61.6 ± 3.0</td>
<td>2.26 ± 0.2</td>
<td>0.12 ± 0.0</td>
<td>5.74 ± 0.1</td>
<td>4.42 ± 0.8</td>
<td>0.99 ± 0.0</td>
<td>0.27 ± 0.0</td>
<td>3.2 ± 0.0</td>
<td>32.5 ± 0.8</td>
<td>1.8 ± 0.1</td>
</tr>
<tr>
<td>11 (Control)</td>
<td>67.0 ± 0.0</td>
<td>2.80 ± 0.0</td>
<td>0.11 ± 0.0</td>
<td>6.35 ± 0.1</td>
<td>4.95 ± 0.2</td>
<td>0.93 ± 0.1</td>
<td>0.31 ± 0.0</td>
<td>0.57 ± 0.0</td>
<td>32.2 ± 0.1</td>
<td>11.2 ± 0.0</td>
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<tr>
<td>11*</td>
<td>57.3 ± 0.8</td>
<td>1.68 ± 0.4</td>
<td>0.12 ± 0.0</td>
<td>5.58 ± 0.3</td>
<td>4.28 ± 1.1</td>
<td>0.87 ± 0.1</td>
<td>0.29 ± 0.1</td>
<td>0.47 ± 0.1</td>
<td>31.0 ± 1.8</td>
<td>12.0 ± 0.7</td>
</tr>
<tr>
<td>13*</td>
<td>65.1 ± 3.2</td>
<td>2.12 ± 0.1</td>
<td>0.12 ± 0.0</td>
<td>6.45 ± 0.1</td>
<td>5.76 ± 0.2</td>
<td>0.93 ± 0.0</td>
<td>0.34 ± 0.0</td>
<td>0.52 ± 0.0</td>
<td>32.2 ± 0.3</td>
<td>12.4 ± 0.3</td>
</tr>
<tr>
<td>33*</td>
<td>64.9 ± 1.5</td>
<td>1.87 ± 0.2</td>
<td>0.13 ± 0.0</td>
<td>15.3 ± 0.1</td>
<td>5.68 ± 0.8</td>
<td>0.95 ± 0.1</td>
<td>0.38 ± 0.1</td>
<td>0.48 ± 0.0</td>
<td>31.5 ± 1.3</td>
<td>31.4 ± 0.4</td>
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<tr>
<td>49*</td>
<td>68.1 ± 1.7</td>
<td>2.43 ± 0.1</td>
<td>0.13 ± 0.0</td>
<td>24.8 ± 0.1</td>
<td>5.62 ± 0.4</td>
<td>0.97 ± 0.0</td>
<td>0.31 ± 0.0</td>
<td>0.50 ± 0.0</td>
<td>33.7 ± 1.1</td>
<td>49.4 ± 0.2</td>
</tr>
<tr>
<td>MMS Site Water</td>
<td>60.9 ± 2.5</td>
<td>1.04 ± 0.4</td>
<td>BD</td>
<td>20.9 ± 0.1</td>
<td>BD</td>
<td>0.22 ± 0.0</td>
<td>0.06 ± 0.0</td>
<td>1.40 ± 0.0</td>
<td>69.6 ± 0.6</td>
<td>14.9 ± 0.1</td>
</tr>
<tr>
<td>(Day 7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>61.3 ± 1.7</td>
<td>0.60 ± 0.0</td>
<td>BD</td>
<td>14.9 ± 1.0</td>
<td>BD</td>
<td>0.11 ± 0.0</td>
<td>0.19 ± 0.0</td>
<td>2.17 ± 0.2</td>
<td>69.7 ± 0.5</td>
<td>6.9 ± 0.8</td>
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<tr>
<td>Ambient Control</td>
<td>58.7 ± 0.7</td>
<td>0.69 ± 0.1</td>
<td>BD</td>
<td>14.3 ± 1.3</td>
<td>BD</td>
<td>0.13 ± 0.0</td>
<td>0.19 ± 0.0</td>
<td>1.16 ± 0.2</td>
<td>70.6 ± 0.9</td>
<td>12.6 ± 2.8</td>
</tr>
<tr>
<td>High</td>
<td>63.9 ± 1.5</td>
<td>0.51 ± 0.0</td>
<td>BD</td>
<td>35.7 ± 9.2</td>
<td>BD</td>
<td>0.12 ± 0.0</td>
<td>0.18 ± 0.0</td>
<td>1.12 ± 0.1</td>
<td>69.1 ± 0.2</td>
<td>32.7 ± 12.3</td>
</tr>
<tr>
<td>Low*</td>
<td>69.5 ± 0.2</td>
<td>0.59 ± 0.0</td>
<td>BD</td>
<td>13.2 ± 1.4</td>
<td>BD</td>
<td>0.17 ± 0.1</td>
<td>0.26 ± 0.0</td>
<td>2.15 ± 0.3</td>
<td>64.4 ± 0.5</td>
<td>6.2 ± 0.5</td>
</tr>
<tr>
<td>Ambient*</td>
<td>67.1 ± 3.8</td>
<td>0.60 ± 0.0</td>
<td>BD</td>
<td>13.0 ± 0.4</td>
<td>BD</td>
<td>0.13 ± 0.0</td>
<td>0.24 ± 0.0</td>
<td>1.11 ± 0.0</td>
<td>68.4 ± 0.9</td>
<td>11.7 ± 0.3</td>
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<tr>
<td>High*</td>
<td>72.3 ± 5.0</td>
<td>0.54 ± 0.0</td>
<td>BD</td>
<td>27.5 ± 1.1</td>
<td>BD</td>
<td>0.15 ± 0.0</td>
<td>0.26 ± 0.0</td>
<td>1.16 ± 0.0</td>
<td>69.8 ± 0.4</td>
<td>23.7 ± 0.1</td>
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<tr>
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<td>BD</td>
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<td>BD</td>
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<td>BD</td>
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<td>0.17 ± 0.0</td>
<td>1.29 ± 0.0</td>
<td>69.9 ± 0.4</td>
<td>12.0 ± 0.3</td>
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<tr>
<td>High</td>
<td>81.0 ± 5.1</td>
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<td>BD</td>
<td>29.0 ± 1.1</td>
<td>BD</td>
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<td>BD</td>
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<td>BD</td>
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<td>0.25 ± 0.0</td>
<td>0.93 ± 0.2</td>
<td>67.9 ± 1.1</td>
<td>12.8 ± 1.9</td>
</tr>
<tr>
<td>High*</td>
<td>82.9 ± 5.0</td>
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<td>BD</td>
<td>24.2 ± 1.1</td>
<td>BD</td>
<td>0.06 ± 0.0</td>
<td>0.46 ± 0.3</td>
<td>1.03 ± 0.0</td>
<td>66.5 ± 2.7</td>
<td>23.5 ± 0.7</td>
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</table>
Table 3) 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.

<table>
<thead>
<tr>
<th></th>
<th>C:N</th>
<th>N:P</th>
<th>C:P</th>
</tr>
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<td></td>
<td></td>
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<tr>
<td><strong>(Day 3)</strong></td>
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<tr>
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<td>6.3 ± 0.2</td>
<td>16.1 ± 0.5</td>
<td>102 ± 5.3</td>
</tr>
<tr>
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<td>6.6 ± 0.3</td>
<td>12.0 ± 0.6</td>
<td>79.2 ± 7.1</td>
</tr>
<tr>
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<td>8.2 ± 0.3</td>
<td>14.8 ± 0.4</td>
<td>122 ± 2.1</td>
</tr>
<tr>
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<td>8.3 ± 0.3</td>
<td>25.5 ± 4.0</td>
<td>212 ± 41</td>
</tr>
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<td>11.1 ± 0.3</td>
<td>23.1 ± 6.1</td>
<td>187 ± 49</td>
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<td>7.9 ± 0.2</td>
<td>15.7 ± 0.7</td>
<td>123 ± 3.2</td>
</tr>
<tr>
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<td>15.7 ± 1.7</td>
<td>125 ± 14</td>
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<td>15.7 ± 1.0</td>
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<td><strong>(Day 10)</strong></td>
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<td>6.6 ± 0.3</td>
<td>12.0 ± 0.6</td>
<td>79.2 ± 7.1</td>
</tr>
<tr>
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<td>13.1 ± 0.5</td>
<td>87.3 ± 3.4</td>
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<td>86.4 ± 2.4</td>
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<td>14.9 ± 0.1</td>
<td>95.2 ± 1.6</td>
</tr>
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<td>12.5 ± 0.7</td>
<td>81.0 ± 4.7</td>
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<tr>
<td>49*</td>
<td>6.4 ± 0.1</td>
<td>14.0 ± 0.4</td>
<td>90.2 ± 3.1</td>
</tr>
<tr>
<td><strong>MMS Site</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td><strong>(Day 7)</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>7.2 ± 0.5</td>
<td>12.8 ± 0.5</td>
<td>92.9 ± 7.3</td>
</tr>
<tr>
<td>Ambient (Control)</td>
<td>7.9 ± 0.2</td>
<td>16.7 ± 0.7</td>
<td>132 ± 3.0</td>
</tr>
<tr>
<td>High</td>
<td>8.0 ± 0.2</td>
<td>16.9 ± 0.8</td>
<td>136 ± 5.9</td>
</tr>
<tr>
<td><strong>(Day 7)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low*</td>
<td>7.3 ± 0.1</td>
<td>16.2 ± 0.8</td>
<td>118 ± 6.2</td>
</tr>
<tr>
<td>Ambient*</td>
<td>7.3 ± 0.0</td>
<td>18.6 ± 0.5</td>
<td>136 ± 4.1</td>
</tr>
<tr>
<td>High*</td>
<td>7.5 ± 0.1</td>
<td>16.5 ± 0.9</td>
<td>124 ± 4.9</td>
</tr>
<tr>
<td><strong>(Day 7)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0°C Treatments</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>8.0 ± 0.2</td>
<td>18.9 ± 2.4</td>
<td>150 ± 17</td>
</tr>
<tr>
<td>Ambient</td>
<td>8.1 ± 0.2</td>
<td>20.9 ± 7.8</td>
<td>170 ± 67</td>
</tr>
<tr>
<td>High</td>
<td>8.4 ± 0.1</td>
<td>21.7 ± 4.2</td>
<td>181 ± 34</td>
</tr>
<tr>
<td><strong>4°C Treatments</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low*</td>
<td>7.7 ± 0.0</td>
<td>18.2 ± 0.2</td>
<td>140 ± 0.7</td>
</tr>
<tr>
<td>Ambient*</td>
<td>7.7 ± 0.1</td>
<td>19.7 ± 0.9</td>
<td>152 ± 8.4</td>
</tr>
<tr>
<td>High*</td>
<td>7.8 ± 0.1</td>
<td>18.8 ± 1.6</td>
<td>146 ± 11</td>
</tr>
</tbody>
</table>
Table 4) 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 colonial *Phaeocystis* cells comprising the final phytoplankton community of each treatment.

<table>
<thead>
<tr>
<th>0°C Treatments</th>
<th>Colonies (mL⁻¹)</th>
<th>Cells (colony⁻¹)</th>
<th>Relative Abundance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>137 ± 94</td>
<td>35 ± 13</td>
<td>93.2</td>
</tr>
<tr>
<td>Ambient (Control)</td>
<td>110 ± 33</td>
<td>29 ± 10</td>
<td>84.3</td>
</tr>
<tr>
<td>High</td>
<td>107 ± 2.0</td>
<td>47 ± 14</td>
<td>88.3</td>
</tr>
<tr>
<td>Low*</td>
<td>59 ± 30</td>
<td>42 ± 26</td>
<td>89.3</td>
</tr>
<tr>
<td>Ambient*</td>
<td>95 ± 18</td>
<td>19 ± 13</td>
<td>76.4</td>
</tr>
<tr>
<td>High*</td>
<td>122 ± 65</td>
<td>36 ± 18</td>
<td>81.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>4°C Treatments</th>
<th>Colonies (mL⁻¹)</th>
<th>Cells (colony⁻¹)</th>
<th>Relative Abundance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>167 ± 42</td>
<td>17 ± 1.7</td>
<td>77.7</td>
</tr>
<tr>
<td>Ambient</td>
<td>164 ± 74</td>
<td>22 ± 1.9</td>
<td>85.5</td>
</tr>
<tr>
<td>High</td>
<td>230 ± 50</td>
<td>24 ± 1.9</td>
<td>90.4</td>
</tr>
<tr>
<td>Low*</td>
<td>700 ± 69</td>
<td>22 ± 17</td>
<td>87.6</td>
</tr>
<tr>
<td>Ambient*</td>
<td>507 ± 12</td>
<td>22 ± 5.6</td>
<td>87.5</td>
</tr>
<tr>
<td>High*</td>
<td>413 ± 151</td>
<td>20 ± 7.7</td>
<td>89.4</td>
</tr>
</tbody>
</table>
Figure 1) Mean chlorophyll $a$ concentrations ± 1 standard deviation of the whole community (>0.7 µm) over the course of the TNB experiment.
Figure 2) Mean bacterial abundance ± 1 standard deviation on (A) Day 3 and (B) Day 10 of the TNB experiment. Bacterial abundance at the start of the experiment (initial community) is also shown (A and B). Letters above each bar indicate whether treatments within a plot are significantly different from each other, and bars that have different letters are significantly different from one another ($p \leq 0.05$).
Figure 3) Mean C absolute uptake rates ± 1 standard deviation of DIC (A, Day 3; B, Day 10) and AA-C (C) for the TNB 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 ≤ 0.05).
Figure 4) Mean N absolute uptake rates ± 1 standard deviation of $\text{NO}_3^-$ (A, Day 3; B, Day 10) and AA-N (C) for the TNB experiment by small (0.7-5.0 µm, white bars) and large (>5.0 µm, black bars) microorganisms. $\text{NO}_3^-$ (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 ($p \leq 0.05$).
Figure 5) 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 MMS experiment.
Figure 6) Mean bacterial abundance ± 1 standard deviation on Day 7 of the TNB experiment. Bacterial abundance at the start of the experiment (initial community) is also shown. Bars that have different letters are significantly different from one another (p ≤ 0.05); data for all treatments in the panel were included in the comparison.
Figure 7) Mean C absolute uptake rates ± 1 standard deviation of 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 ≤ 0.05); all data for treatments within a single panel (i.e. A or B) were included in the comparison.
Figure 8) Mean N absolute uptake rates ± 1 standard deviation of NO$_3^-$ (A) and AA-N (B) for the MMS 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 ≤ 0.05); all data for treatments within a single panel (i.e. A or B) were included in the comparison.
Figure 9) MDS plot based on Bray-Curtis similarities of the average phytoplankton community composition of each treatment. Color indicates nutrient supply ratios with blue indicating low N:P, green indicating ambient N:P, and red indicating high N:P. Treatments that received Fe are specified with a * sign. Circles indicate treatments at 0°C, while triangles indicate treatments at 4°C. Additionally, treatments at the same temperature are separated by the dotted line.
Figure 10) Mean relative abundances of prominent phytoplankton groups other than colonial *Phaeocystis* cells for each treatment at the end of the experiment.
Table S1) Mean particulate concentrations ± 1 standard deviation (n=3) of the whole community (>0.7 µm) collected from each site (TNB and MMS), and mean particulate concentrations of the communities from each treatment measured on Day 3 and Day 10 for TNB, and on Day 7 for MMS. Concentrations are in units of µmol C, N, or P L⁻¹.

<table>
<thead>
<tr>
<th>Particulate C µmol C L⁻¹</th>
<th>Particulate N µmol N L⁻¹</th>
<th>Particulate P µmol P L⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TNB Site</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2*</td>
<td>62.7 ± 1.4</td>
<td>7.6 ± 0.2</td>
</tr>
<tr>
<td>11 (Control)</td>
<td>64.8 ± 0.4</td>
<td>7.8 ± 0.2</td>
</tr>
<tr>
<td>11*</td>
<td>63.0 ± 3.3</td>
<td>7.8 ± 0.1</td>
</tr>
<tr>
<td>13*</td>
<td>65.1 ± 0.6</td>
<td>8.3 ± 0.3</td>
</tr>
<tr>
<td>33*</td>
<td>66.1 ± 1.4</td>
<td>8.3 ± 0.2</td>
</tr>
<tr>
<td>49*</td>
<td>68.9 ± 5.8</td>
<td>8.6 ± 0.1</td>
</tr>
<tr>
<td><strong>TNB (Day 10)</strong></td>
<td>53.4 ± 3.9</td>
<td>8.1 ± 0.2</td>
</tr>
<tr>
<td>11 (Control)</td>
<td>50.2 ± 0.8</td>
<td>7.5 ± 0.1</td>
</tr>
<tr>
<td>11*</td>
<td>56.8 ± 1.9</td>
<td>8.9 ± 0.4</td>
</tr>
<tr>
<td>13*</td>
<td>58.8 ± 1.3</td>
<td>9.2 ± 0.1</td>
</tr>
<tr>
<td>33*</td>
<td>54.0 ± 1.5</td>
<td>8.3 ± 0.2</td>
</tr>
<tr>
<td>49*</td>
<td>54.3 ± 2.2</td>
<td>8.4 ± 0.3</td>
</tr>
<tr>
<td><strong>MMS Site</strong></td>
<td>22.8 ± 1.9</td>
<td>3.2 ± 0.1</td>
</tr>
<tr>
<td><strong>MMS (Day 7)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>53.3 ± 5.8</td>
<td>6.7 ± 0.8</td>
</tr>
<tr>
<td>Ambient (Control)</td>
<td>52.4 ± 1.0</td>
<td>6.5 ± 0.1</td>
</tr>
<tr>
<td>High</td>
<td>50.8 ± 3.2</td>
<td>6.3 ± 0.4</td>
</tr>
<tr>
<td>Low*</td>
<td>64.0 ± 8.6</td>
<td>8.8 ± 1.3</td>
</tr>
<tr>
<td>Ambient*</td>
<td>64.2 ± 2.1</td>
<td>8.8 ± 0.2</td>
</tr>
<tr>
<td>High*</td>
<td>62.7 ± 3.6</td>
<td>8.3 ± 0.6</td>
</tr>
<tr>
<td>Low</td>
<td>61.0 ± 4.2</td>
<td>7.7 ± 0.4</td>
</tr>
<tr>
<td>Ambient</td>
<td>55.7 ± 5.1</td>
<td>6.9 ± 0.7</td>
</tr>
<tr>
<td>High</td>
<td>57.2 ± 5.4</td>
<td>6.9 ± 0.8</td>
</tr>
<tr>
<td>Low*</td>
<td>79.0 ± 3.4</td>
<td>10.3 ± 0.4</td>
</tr>
<tr>
<td>Ambient*</td>
<td>73.4 ± 5.1</td>
<td>9.5 ± 0.6</td>
</tr>
<tr>
<td>High*</td>
<td>77.2 ± 3.8</td>
<td>9.9 ± 0.6</td>
</tr>
</tbody>
</table>
Table S2) Changes ($\Delta$) in $\text{NO}_3^-$ concentrations for the MMS treatments where the N:P ratio was elevated. Values were obtained by calculating the difference between concentrations measured on Days 0, 3, 5, and 7. (Day 0 samples for the the 0°C High treatment were not measured so this value was unable to be determined; ND)

<table>
<thead>
<tr>
<th>Days</th>
<th>Treatment</th>
<th>$\Delta \text{NO}_3^-$</th>
<th>μmol N L$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 3</td>
<td>0°C High</td>
<td></td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>0°C High*</td>
<td></td>
<td>-4.6</td>
</tr>
<tr>
<td></td>
<td>4°C High</td>
<td></td>
<td>-4.4</td>
</tr>
<tr>
<td></td>
<td>4°C High*</td>
<td></td>
<td>-6.8</td>
</tr>
<tr>
<td>3 - 5</td>
<td>0°C High</td>
<td></td>
<td>-0.3</td>
</tr>
<tr>
<td></td>
<td>0°C High*</td>
<td></td>
<td>-0.3</td>
</tr>
<tr>
<td></td>
<td>4°C High</td>
<td></td>
<td>-0.4</td>
</tr>
<tr>
<td></td>
<td>4°C High*</td>
<td></td>
<td>-1.2</td>
</tr>
<tr>
<td>5 - 7</td>
<td>0°C High</td>
<td></td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>0°C High*</td>
<td></td>
<td>-2.3</td>
</tr>
<tr>
<td></td>
<td>4°C High</td>
<td></td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>4°C High*</td>
<td></td>
<td>-2.2</td>
</tr>
</tbody>
</table>
CHAPTER 5

UPTAKE OF CARBON, NITROGEN, AND PHOSPHORUS UNDER A MATRIX OF TEMPERATURE AND IRON BY THREE ANTARCTIC PHYTOPLANKTON SPECIES (FRAGILARIOPSIS CYLINDRUS, PSEUDO-NITZSCHIA SUBCURVATA, AND PHAEOCYSTIS ANTARCTICA)

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ABSTRACT: In the next century, sea surface temperatures in the Southern Ocean and iron input, which typically limits phytoplankton growth in this region, are predicted to increase. Quantifying the sensitivity of phytoplankton to these parameters is essential for predicting how nutrient cycles may change in the future. We used stable isotope tracer techniques and radiolabeling to measure uptake rates of inorganic and organic carbon, nitrogen, and phosphorus substrates by three different phytoplankton species—two pennate diatoms (*Fragilariopsis cylindrus* and *Pseudo-nitzschia subcurvata*) and a haptophyte (*Phaeocystis antarctica*) under a matrix of temperature (0°C and 4°C) and iron (1 nmol L$^{-1}$ and 500 nmol L$^{-1}$) conditions. We found that the response of the phytoplankton to these conditions varied between species, and that patterns were not consistent between substrates. We also found that elemental stoichiometry—both particulate ratios and nutrient uptake ratios—were impacted by temperature and iron. Our data indicate that when temperature is elevated pennate diatoms acquire nutrients more rapidly than *P. antarctica*, and that carbon overconsumption and an increased demand for nitrogen at higher temperature results in changes to nutrient ratios. These results indicate that shifts in the structure of the Antarctic phytoplankton community may occur in the future, and that the stoichiometry of the Southern Ocean may change if current predictions about temperature and iron hold.
INTRODUCTION

The Southern Ocean plays a critical role in the global climate system and the biogeochemical cycling of key elements such as carbon, nitrogen, and phosphorus (Arrigo et al. 1999; Sarmiento et al. 2004). It also directly regulates Earth’s climate through both physical and biological mechanisms. As atmospheric carbon dioxide (CO₂) dissolves into the surface waters, it can be removed to the deep ocean through currents or taken up by phytoplankton for photosynthesis (Sabine et al. 2004). In the Southern Ocean, large phytoplankton blooms occur during the austral spring and summer when light is available, resulting in seasonally high rates of primary production (Arrigo et al. 2008; Smith & Comiso 2008). The high rates of primary production support a diverse assemblage of higher trophic level organisms (Woehler 1993; Ainley 1985; Ainley et al. 2017), and the fixed carbon has the potential to sink to the deep ocean via the biological pump, where it can be sequestered (Broecker 1991; Sabine et al. 2004; Siegel et al. 2014).

The ecological and biogeochemical balance of the Southern Ocean may be disrupted by rising sea surface temperatures, which are currently increasing at much higher rates in comparison to Earth’s geologic past (IPCC 2014). In certain regions of the Southern Ocean, like the Western Antarctic Peninsula, considerable changes due to sea surface warming have already been witnessed (Montes-Hugo et al. 2009; Constable et al. 2014 and refs therein), and sea surface temperatures are predicted to continue to rise by approximately 3 - 4°C by 2100 (IPCC 2014). The concentration of iron, a micronutrient that typically limits phytoplankton growth in the Southern Ocean (Martin 1990), is expected to increase due to several climate change-driven mechanisms.
Glaciers and icebergs contain bioavailable iron, and as they melt, due to higher temperatures, the iron in the melt water is delivered to the ocean (Sedwick & DiTullio 2007; Raiswell et al. 2008). Additionally, changing wind patterns may increase the eolian supply of iron that the Southern Ocean receives (Revel-Rolland et al. 2006). With more iron available for growth, the rates at which other dissolved nutrients, including carbon, nitrogen and phosphorus, are taken up by the phytoplankton may change.

Elevated sea surface temperatures and higher iron concentrations could have major implications for primary production, carbon export, and the Southern Ocean ecosystem as a whole. The Southern Ocean supports a diverse microbial community that includes diatoms, haptophytes, dinoflagellates, and picoplankton (Arrigo et al. 1999; Wolf et al. 2013; Wright et al. 2010), and studies show that certain species of phytoplankton may be better suited to higher temperatures and/or able to acquire iron more efficiently (Rose et al. 2009; Feng 2010; Xu et al. 2014; Zhu et al. 2016). It is important to determine how different phytoplankton species respond to elevated temperatures and higher concentrations of iron, because the efficacy of the biological pump depends on the species present, with larger, heavier cells generally sinking to depth more rapidly than smaller cells and, thus, exporting more carbon (Dugdale & Wilkerson 1998; Falkowski et al. 1998; Jin et al. 2006). Furthermore, elemental stoichiometry varies between phytoplankton species (Planavsky 2014), and since the Southern Ocean exerts a strong influence on global nutrient inventories through the formation of deep water (Sarmiento et al. 2004), understanding which species and/or groups of phytoplankton will be at a competitive advantage in the future will allow us to better predict how biogeochemical cycles are likely to change.
To assess how elevated temperature and iron concentrations may impact the physiology of Southern Ocean phytoplankton and nutrient cycles, we conducted experiments on three important Southern Ocean phytoplankton species, two different pennate diatoms, *Fragilariopsis cylindrus* and *Pseudo-nitzschia subcurvata*, and the haptophyte, *Phaeocystis antarctica*. *F. cylindrus* is typically the most abundant diatom in water column assemblages collected from Antarctic ice edge zones (Kang & Fryxell 1992), and recent research on *Pseudo-nitzschia* spp. demonstrates the potential of this genus to become more prevalent at warmer temperatures (Boyd et al. 2016; Hutchins & Boyd 2016). *P. antarctica* is a haptophyte that typically dominates the early phases of the annual bloom cycle (Smith et al. 2000). For our experiment, we used cultures that had been recently (i.e. ~16 months) isolated from the Ross Sea, Antarctica. Cultures were incubated for an extended period of time (8 weeks) under a matrix of temperature and iron with the goal of determining how these conditions affect uptake rates of inorganic and organic carbon, nitrogen, and phosphorus substrates and the elemental stoichiometry of the different species.

**MATERIALS AND METHODS**

*Field Collection and Culture Conditions*

Seawater was collected from the Ross Sea (77.62° S, 165.47° E) in January and February 2013 and used to prepare unialgal cultures of two pennate diatom species, *Fragilariopsis cylindrus* and *Pseudo-nitzschia subcurvata*, and the haptophyte, *Phaeocystis antarctica*. The isolates were cultured using 0.2 µm-filtered Ross Sea seawater. The seawater was collected from the same location as the phytoplankton
species using trace metal clean techniques. The stock cultures were transported to the University of Southern California, and measures were taken to minimize disruption to the cultures during transport. Cultures were maintained at 0°C in an incubator and provided with continuous light using white fluorescent bulbs that emitted 80 µmol photons m\(^{-2}\) s\(^{-1}\). The irradiance level was selected to mimic light levels found in the Ross Sea at approximately 10-20 m, where both diatoms and *P. antarctica* are commonly found (Long 2010). The Ross Sea seawater used as culture diluent was enriched with trace nutrients and vitamins according to the Aquil recipe (Sunda et al. 2005).

**Experimental Procedure**

Treatments were set up by dividing the stock cultures into triplicate 500 mL acid-washed polycarbonate bottles and performing experimental manipulations. Cultures were grown for eight weeks (approximately ≥ 16 generations for all treatments) under a matrix of two temperature levels (0°C and 4°C) and two iron levels (1 nmol L\(^{-1}\) and 500 nmol L\(^{-1}\); additions were made using iron (III) chloride, FeCl\(_3\)). These iron levels were selected to match a previous culture study looking at the effects of iron deplete and iron replete conditions on Antarctic phytoplankton (Xu et al. 2014). The 0C-Fe is the control, and treatments will be referred to as 0C+Fe, 4C-Fe, and 4C+Fe hereafter. The FeCl\(_3\) that was added to the treatments was chelated with ethylenediaminetetraacetic acid (EDTA, final medium concentration 50 µmol L\(^{-1}\)). Treatments continued to receive the same amount of light (80 µmol photons m\(^{-2}\) s\(^{-1}\)) as the stock cultures. The filtered seawater diluent was collected in late-ustral summer such that the concentrations of macronutrients, including nitrate (NO\(_3^\text{-}\)) and phosphate (PO\(_4^{3-}\)) were relatively depleted. Therefore, NO\(_3^\text{-}\) and PO\(_4^{3-}\) were added to the diluent to a final concentration of ~50.0 µmol N L\(^{-1}\) and ~5.0 µmol P.
L$^{-1}$ respectively (Table 1). NO$_3^-$ and PO$_4^{3-}$ were added at a ratio lower than Redfield proportions to match conditions typically observed in the Southern Ocean (Martiny et al. 2013). Silicate (Si) was present in the diluent at a concentration of ~68.0 µmol Si L$^{-1}$.

Following experimental manipulations, cultures were diluted every 2 days using diluent that had been temperature adjusted to either 0°C or 4°C. Dilution volumes were selected such that the in vivo chlorophyll $a$ fluorescence of each replicate bottle had reached the pre-dilution fluorescence value. This allowed each bottle to achieve steady-state exponential growth. Sampling occurred after growth rates (approximately 0.2 – 0.8 day$^{-1}$; presented in Zhu et al. 2016) had remained stable for three to five consecutive dilutions. On the day of the experiment, samples to measure biomass and nutrient concentrations were collected. Samples for nutrients were filtered through Whatman glass fiber filters (GF/F; nominal pore size of 0.7 µm; combusted for 2 hours at 450°C) and the filtrate was collected.

Experiments were also set up to measure uptake rates of carbon, nitrogen, and phosphorus substrates. Stable isotope tracer techniques using $^{13}$C-labeled carbon and $^{15}$N-labeled nitrogen were used to measure uptake of carbon and nitrogen, while radiolabeling methods using $^{33}$P-labeled phosphorus were used to determine uptake of phosphorus. Incubations to measure carbon and nitrogen uptake rates were completed in acid-washed (10% HCl; trace metal grade) 60 mL PETG bottles. Acid-washed 30 mL PETG bottles were used to measure phosphorus uptake. Additions of either $^{13}$C-labeled carbon substrates or $^{15}$N-labeled nitrogen substrates were performed at an estimated 10% enrichment above background concentrations (atom % enrichments ranged from 5.8 – 9.2% for inorganic nutrient substrates, and from 9.5 – 35.2% for organic substrates), and
incubations lasted for approximately 4 hours at both temperature levels. Carbon and nitrogen substrates included $^{13}$C-labeled sodium bicarbonate, (NaH$^{13}$CO$_3$; 99%), $^{15}$N-labeled ammonium chloride ($^{15}$NH$_4$Cl; 98.85%), $^{15}$N-labeled potassium nitrate (K$^{15}$NO$_3$; 98%), dual $^{13}$C- and $^{15}$N-labeled urea (98%) and a dual-labeled algal amino acid mixture comprised of 16 amino acids, specified as either AA-C or AA-N (97-99%); all labeled substrates came from Cambridge Isotopes Laboratories, Andover, MA. Uptake of $^{13}$C-labeled bicarbonate, referred to as DIC uptake henceforth, was measured in the same incubations that received additions of $^{15}$N-labeled NO$_3$- and $^{15}$N-labeled ammonium (NH$_4^+$). At the end of the incubations, phytoplankton cells were collected onto GFF filters and stored frozen (-40°C) until analysis on an isotope ratio mass spectrometer.

Phosphorus substrates included H$_3^{33}$PO$_4$ and adenosine triphosphate ($\gamma$-AT$^{33}$P). Bottles received additions of H$_3^{33}$PO$_4$ (40-158 Ci mg$^{-1}$) and $\gamma$-AT$^{33}$P (3000 Ci mmol$^{-1}$; substrates came from PerkinElmer). Incubations lasted for approximately 1 hour after which they were filtered onto GFF filters. Filters were rinsed with 0.2 µm filtered seawater to remove any external phosphorus and placed in scintillation cocktail. Radioactivity of the filters was determined using a liquid scintillation counter (Beckman LS 6000 Series).

**Uptake Rates**

All cultures were in steady-state exponential growth at the time rates were measured, and growth rates varied between the species and the treatments (results presented in Zhu et al. 2016). Uptake rates for all substrates are presented as both absolute rates and as uptake rates per cell; we have also included specific uptake rates
(h⁻¹) in Supplementary Materials (Figures S1, S2, S3). We focus on absolute uptake rates
because uptake rates calculated using stable isotopes and radioactive isotopes are most
easily compared to each other when absolute rates are used (Collos & Slawyk 1985).
Using absolute rates also incorporates the differences in biomass that were a result of
temperature and/or iron treatments. Furthermore, absolute rates are expressed as µmol C,
N, or P L⁻¹ h⁻¹, which make it possible to calculate uptake rates per cell as well as
stoichiometric uptake ratios. Rates for all substrates were calculated per cell by dividing
the absolute rate by the number of cells present within each treatment replicate.

Carbon and nitrogen absolute uptake rates (ρ) were calculated using the equations
described in Hama et al. (1983), Bronk et al. (2014), and Dugdale & Wilkerson (1986):

\[
ρ = \frac{P_X \text{ at} %xs \times [PX]}{SP \text{ at} %xs \times T}
\]

where \(P_X \text{ at} %xs\) is either the \(^{13}\)C or \(^{15}\)N atom % enrichment of the particulates minus 1.08
(the natural \(^{13}\)C enrichment of phytoplankton; Slawyk et al. 1977; Lefebvre et al. 2012) in
the case of carbon, or 0.366 (\(^{15}\)N atom % enrichment of an atmospheric N\(_2\) gas standard)
for nitrogen. \(SP \text{ at} %xs\) is the initial enrichment of the substrate Source Pool minus either
the \(^{13}\)C or \(^{15}\)N enrichment of the standard. \([PX]\) is the concentration (µmol X L⁻¹) of
either particulate carbon or particulate nitrogen post incubation and T is the incubation
time. Specific uptake rates (V) of carbon and nitrogen were calculated using the same
equation that was used to calculate the absolute rate except the \([PX]\) term was excluded.

Absolute phosphorus uptake rates were calculated using the following equation
modified from Fu et al. (2005).

\[
ρ = \frac{R_{\text{SAMPLE}} \times [P]}{A \times T}
\]
where \( R_{\text{SAMPLE}} \) is the radioactivity on the filter, and \( A \) is the total amount of radioactivity added and available for uptake. \([P]\) is the ambient concentration of the P source (either \( \text{PO}_4^{3-} \) or ATP), and \( T \) is the incubation time. For ATP, concentrations were assumed to be 0.001 \( \mu \text{mol} \text{ P L}^{-1} \). This value is the approximate average of ATP measured in the Ross Sea in December and January (Knox 1986). Specific uptake rates for phosphorus compounds were calculated by multiplying the absolute rate by the concentrations of particulate phosphorus.

**Nutrient and Biomass Analysis**

A Lachat QuickChem 8500 autoanalyzer was used to measure concentrations of \( \text{NO}_3^- \), \( \text{PO}_4^{3-} \), and Si in duplicate (Parsons et al. 1984). Concentrations of \( \text{NH}_4^+ \) were measured in triplicate on a Shimadzu UV-1601 spectrophotometer using the phenol-hypochlorite method (Koroleff 1983). Concentrations of urea were measured manually using the diacetyl monoxime thiosemicarbazide method (Price and Harrison 1987). Dissolved primary amine (DPA) samples were measured on a Shimadzu RF-1501 spectrofluorometer after being prepared in triplicate using the OPA (o-phthaldialdehyde) method (Parsons et al. 1984). A Shimadzu 5000A TOC-V/TNM was used to measure total dissolved nitrogen (TDN) and dissolved organic carbon (DOC) (Sharp et al. 2004; Hansell 2005). Dissolved organic nitrogen (DON) was determined by calculating the difference between TDN and the sum of the inorganic nitrogen species; propagation of error was used to calculate DON standard deviation. Particulate carbon and particulate nitrogen concentrations were measured on a Europa 20/20 isotope ratio mass spectrometer at the same time that the \(^{13}\text{C} \) and \(^{15}\text{N} \) atom % enrichments of the samples were measured. Total particulate phosphorus was analyzed by baking a GF/F filter for 2
hours at 550°C followed by acid extraction, and subsequent measurement of the concentration of orthophosphate in the extract colorimetrically (Aspila & Agemian 1976). Chlorophyll $a$ concentrations were measured on a Turner Design Model 10-AU fluorometer (Parsons et al. 1984). Cell counts were obtained microscopically using the methods described in Xu et al. (2014).

**Statistical Analysis**

The open source RStudio statistical program version 0.99.490 (Rstudio Team 2015) was used for data analysis. Data were checked for normality and homogeneity of variance and were log transformed prior to statistical analysis if they were not normally distributed. A one-way analysis of variance (ANOVA) was used to determine if there were significant differences between the different treatments. Post hoc Tukey’s Tests were used to locate means that were significantly different from one another. If a p value was less than 0.05, the means were considered to be significantly different from one another.

**RESULTS**

**Biomass and Nutrients**

For *F. cylindrus* and *P. antarctica*, treatments with iron had higher concentrations of chlorophyll $a$ than those treatments without iron ($p < 0.002$; Table 1). For *P-n. subcurvata*, treatments at 4°C had higher concentrations of chlorophyll $a$ than treatments at 0°C ($p < 0.05$), however, abundances of cells were highest in treatments without iron ($p < 0.003$). In both diluent types, DON concentrations were highest (~90 µmol N L$^{-1}$) followed by NO$_3^-$ (~50 µmol N L$^{-1}$) and NH$_4^+$ (~2.0 µmol N L$^{-1}$); concentrations of urea and DPA were both less than 1 µmol N L$^{-1}$. Trends for concentrations of NO$_3^-$ and PO$_4^{3-}$
were similar to the patterns observed for concentrations of chlorophyll $a$. Concentrations of NO$_3^-$ and PO$_4^{3-}$ were lower in treatments with iron for *F. cylindrus* and *P. antarctica*. For *P-n. subcurvata*, concentrations of NO$_3^-$ and PO$_4^{3-}$ were lower in treatments at 4°C.

**Absolute Carbon Uptake**

The influence of elevated temperature and iron on absolute DIC uptake rates ($\rho_{\text{DIC}}$) varied between phytoplankton species (Figure 1A). For *F. cylindrus* temperature elevation and iron-replete conditions, individually and in combination, resulted in significantly higher $\rho_{\text{DIC}}$ compared to the 0C-Fe control ($p < 0.0001$). $\rho_{\text{DIC}}$ in the 4C+Fe treatment was highest, nearly triple that of the 0C-Fe control, and significantly higher than in the 0C+Fe and 4C-Fe treatments ($p < 0.0001$). For *P-n. subcurvata*, temperature and iron also influenced $\rho_{\text{DIC}}$. Compared to the 0C-Fe control, $\rho_{\text{DIC}}$ in the 0C+Fe treatment was lower ($p < 0.0001$), and $\rho_{\text{DIC}}$ in the 4C-Fe and 4C+Fe treatments were higher ($p < 0.001$). $\rho_{\text{DIC}}$ of the 4C-Fe treatment was also significantly higher than the 4C+Fe treatment ($p < 0.0001$). For *P. antarctica*, $\rho_{\text{DIC}}$ in all treatments were significantly higher ($p < 0.0001$) than in the 0C-Fe control, however, the magnitude of increase in the iron-replete treatments was much higher, and rates in the 0C+Fe and 4C+Fe were higher than the 4C-Fe treatment ($p < 0.0001$).

In comparison to DIC, absolute uptake rates of AA-C ($\rho_{\text{AA-C}}$) were less influenced by elevated temperature and iron (Figure 1B). For both *F. cylindrus* and *P. antarctica* none of the treatments had significantly different $\rho_{\text{AA-C}}$ than the 0C-Fe controls. For *P-n. subcurvata*, $\rho_{\text{AA-C}}$ in the 4C+Fe treatment was higher than both the 0C-Fe control and 0C+Fe treatment ($p < 0.002$), but not significantly different from 4C-Fe.
Absolute uptake rates of urea-C (ρ\textsubscript{UREA-C}) were not detected for either \textit{F. cylindrus} or \textit{P. antarctica} (i.e., measured PC atom % were either \(\leq 1.08\), the natural \(^{13}\text{C}\) enrichment of phytoplankton, resulting in uptake rates that were either \(\leq 0.00\)). For \textit{P-n. subcurvata}, ρ\textsubscript{UREA-C} was only detected in the 0C-Fe control and 4C-Fe treatments as 0.6 ± 0.5 nmol C L\(^{-1}\) h\(^{-1}\) and 2.8 ± 2.2 nmol C L\(^{-1}\) h\(^{-1}\) respectively. These values were an order of magnitude smaller than ρ\textsubscript{AA-C}.

**Absolute Nitrogen Uptake**

The effects of temperature and iron on nitrogen uptake varied between phytoplankton species and between nitrogen substrates (Figure 2). For \textit{F. cylindrus}, significant increases in uptake rates of inorganic nitrogen substrates occurred, while uptake rates of organic nitrogen substrates were not impacted. Compared to the 0C-Fe control, absolute uptake rates of NH\(_4^+\) (ρ\textsubscript{NH4}) increased in the 4C-Fe treatment (\(p < 0.001\); Figure 2A), and absolute uptake rates of NO\(_3^-\) (ρ\textsubscript{NO3}) increased in all treatments (\(p < 0.0001\); Figure 2B). Highest ρ\textsubscript{NO3} occurred at 4°C, and both the 4C-Fe and 4C+Fe treatments had higher ρ\textsubscript{NO3} than the treatments at 0°C (\(p < 0.04\)). Significant changes were not detected for absolute uptake rates of urea (ρ\textsubscript{UREA-N}) or AA-N (ρ\textsubscript{AA-N}) by \textit{F. cylindrus} (Figure 2C and D).

For \textit{P-n. subcurvata}, ρ\textsubscript{NH4}, ρ\textsubscript{NO3}, and ρ\textsubscript{UREA-N} generally followed the same pattern (Figure 2A, B, and C). Treatments that had high iron concentrations (0C+Fe and 4C+Fe) had lower uptake rates than those treatments with low iron concentrations (0C-Fe and 4C-Fe; \(p < 0.006\)). Examining ρ\textsubscript{NO3} in more detail, temperature elevation increased uptake rates. ρ\textsubscript{NO3} of the 4C-Fe treatment was significantly higher than the 0C-Fe control, and ρ\textsubscript{NO3} of the 4C+Fe treatment was significantly higher than the 0C+Fe
treatment (p < 0.0001; Figure 2B). \( \rho_{AA-N} \) by *P-n. subcurvata* was not impacted with the exception of the 4C+Fe treatment, which had significantly higher \( \rho_{AA-N} \) than both the 0C-Fe control and 0C+Fe treatment (p < 0.0001; Figure 2D). This is the same trend that was observed for \( \rho_{AA-C} \) (Figure 1B).

For *P. antarctica*, \( \rho_{NH4} \) and \( \rho_{UREA-N} \) exhibited the same pattern (Figure 2A and C). Compared to the 0C-Fe control, \( \rho_{NH4} \) and \( \rho_{UREA-N} \) increased (p < 0.04) in the 0C+Fe treatments, and the other treatments were not impacted. For NO\(_3\), \( \rho_{NO3} \) of all treatments were higher than \( \rho_{NO3} \) measured in the 0C-Fe control (p < 0.0001; Figure 2B). \( \rho_{NO3} \) in the 0C+Fe treatment was the highest followed by 4C+Fe and lastly the 4C-Fe treatment, and all of these treatments had \( \rho_{NO3} \) that were significantly different from one another (p < 0.003). \( \rho_{AA-N} \) was not impacted (Figure 2D), which was the same trend observed for \( \rho_{AA-C} \) (Figure 1B).

**Absolute Phosphorus Uptake**

Compared to the 0C-Fe control, absolute uptake rates of \( \rho_{PO4-3} \) (\( \rho_{PO4} \)) by *F. cylindrus* increased in the 0C+Fe treatment and decreased in both the 4C-Fe and 4C+Fe treatments (Figure 3A). These changes were not significant, however, both the 4C-Fe and 4C+Fe treatments were significantly lower than the 0C+Fe treatment (p < 0.003). Compared to the other treatments, \( \rho_{PO4} \) by *P-n. subcurvata* was significantly lower in the 0C+Fe treatment (p < 0.03). For *P. antarctica*, the treatments with high concentrations of iron had significantly higher \( \rho_{PO4} \) than the 0C-Fe control (p < 0.02).

Absolute uptake of ATP (\( \rho_{ATP} \)) by the three species did not follow the same trends as \( \rho_{PO4-3} \) (Figure 3B). For *F. cylindrus*, \( \rho_{ATP} \) was higher (p < 0.002) in the 0C+Fe treatment and lower (p < 0.03) in the 4C-Fe treatment compared to rates in the 0C-Fe
control and 4C+Fe treatment. For *P-n subcurvata*, all treatments had higher $\rho_{\text{ATP}}$ than the 0C-Fe control ($p < 0.04$). The highest $\rho_{\text{ATP}}$ was measured in the 4C+Fe treatment, and this treatment was also significantly higher than the 0C+Fe and the 4C-Fe treatment ($p < 0.007$). $\rho_{\text{ATP}}$ by *P. antarctica* was not impacted with the exception of the 4C-Fe treatment, which had significantly lower rates than the other treatments ($p < 0.001$).

**Cellular Uptake Rates**

Trends in uptake rates calculated per cell largely mirrored absolute uptake rates. However, in some cases the trends were completely different (Table 2). This was particularly evident for *P-n subcurvata*, where cellular uptake rates for many of the substrates in the iron-replete treatments were not significantly different from the 0C-Fe control. This was different from absolute uptake rates where iron addition resulted in significantly lower rates for several substrates. For *P. antarctica*, fewer statistical differences were detected for cellular uptake rates compared to absolute uptake rates. For $\text{NH}_4^+$, $\text{NO}_3^-$, urea, and $\text{PO}_4^{3-}$ absolute uptake rates in the 0C+Fe treatment were significantly higher than the 0C-Fe control, however, this difference was not observed for cellular uptake rates.

**Particulate Elemental Ratios**

Elemental ratios for particulate carbon to nitrogen (C:N), carbon to phosphorus (C:P), and nitrogen to phosphorus (N:P) varied between species (Figure 4; Table 3). For *F. cylindrus* C:N ratios were lower in all treatments in comparison to the 0C-Fe control ($p < 0.0001$). A similar trend was found for *P. antarctica*. C:N ratios decreased ($p < 0.002$) in the 0C+Fe and 4C+Fe treatments, but were not impacted in the 4C-Fe treatment. For
*P*-n *subcurvata*, C:N ratios decreased (p < 0.001) in the 0C+Fe treatment, however, in both of the temperature treatments (4C-Fe and 4C+Fe) the C:N ratio increased (p < 0.02).

Trends for particulate C:P and N:P ratios did not have the same pattern as C:N ratios (Figure 4; Table 3). In comparison to the 0C-Fe control, C:P ratios of the two diatom species did not change with the exception of the 4C-Fe treatment for *F. cylindrus*, which significantly decreased (p < 0.0001). For *P. antarctica* C:P ratios significantly decreased in the 0C+Fe treatment, but significantly increased in the 4C-Fe and 4C+Fe treatments (p < 0.0001). Displaying the same trend as C:P ratios, particulate N:P ratios of *P-n. subcurvata* were also not affected by temperature and iron changes. Particulate N:P ratios of *F. cylindrus* ratios increased in the high iron treatments at both 0°C and 4°C (p < 0.0001), and N:P ratios for *P. antarctica* increased in the 4C-Fe and 4C+Fe treatments (p < 0.0001).

**Uptake Ratios**

Trends for uptake ratios of DIC to NO$_3^-$ ($\rho_{\text{DIC}:\text{NO}_3}$), DIC to PO$_4^{3-}$ ($\rho_{\text{DIC}:\text{PO}_4}$), and NO$_3^-$ to PO$_4^{3-}$ ($\rho_{\text{NO}_3:\text{PO}_4}$) also varied between species (Figure 5; Table 3). Uptake ratios for both *F. cylindrus* and *P. antarctica* did not follow the same patterns as particulate ratios (Table 3). For *F. cylindrus* and *P. antarctica*, $\rho_{\text{DIC}:\text{NO}_3}$ ratios increased (p < 0.04) in all treatments compared to the 0C-Fe control. The 4C-Fe treatment for *F. cylindrus* was the exception to this trend where no change was observed. In both of the high temperature treatments, $\rho_{\text{DIC}:\text{PO}_4}$ ratios increased for *F. cylindrus* (p < 0.04), while $\rho_{\text{DIC}:\text{PO}_4}$ ratios increased in the high iron treatments for *P. antarctica* (p < 0.0001). For *F. cylindrus*, $\rho_{\text{NO}_3:\text{PO}_4}$ ratios increased in the 4C-Fe and 4C+Fe treatments (p < 0.003), following the same trend as $\rho_{\text{DIC}:\text{PO}_4}$. For *P. antarctica*, $\rho_{\text{NO}_3:\text{PO}_4}$ ratios did not change.
Uptake ratio trends for *P-n subcurvata* followed the same pattern as particulate ratios (Table 3), where $\rho_{\text{DIC}}:\rho_{\text{PO4}}$ and $\rho_{\text{NO3}}:\rho_{\text{PO4}}$ were not affected by temperature and iron, but $\rho_{\text{DIC}}:\rho_{\text{NO3}}$ decreased in the 0C+Fe treatment ($p < 0.04$) and increased in the 4C-Fe and 4C+Fe treatments ($p < 0.0001$).

**DISCUSSION**

*Effect of Temperature and Iron on Inorganic Nutrient Uptake*

The effects of temperature and iron differed among the three species; however, certain trends were evident. Temperature elevation and iron-replete conditions generally resulted in higher absolute uptake rates of inorganic nutrients ($\rho_{\text{DIC}}, \rho_{\text{NH4}}, \rho_{\text{NO3}}, \rho_{\text{PO4}}$). This result is consistent with results from the majority of unialgal culture studies showing that when temperature is elevated, carbon fixation and growth rates increase to a threshold that is species specific (Eppley 1972; Verity 1982; Boyd et al. 2013; Coello-Camba & Agusti 2017). This is also usually the case when microorganisms are relieved of iron stress (Martin 1990; Cassar et al. 2012; Kwon et al. 2014). Within the general model of absolute uptake rates increasing as a function of elevated temperature and iron addition, differences existed between the three species and the substrates.

For *F. cylindrus*, our results for $\rho_{\text{DIC}}, \rho_{\text{NH4}},$ and $\rho_{\text{NO3}}$ indicate that both temperature and iron have the potential to increase absolute uptake rates with an additive effect of the two variables for $\rho_{\text{DIC}}$ (Figure 1A). The combined effect of temperature elevation and iron addition has previously been observed. *F. cylindrus* growth rates increased in a study assessing the impacts of several global change parameters of which temperature and iron were included (Xu et al. 2014), and the relative abundance of *Fragilariopsis* spp.
increased in a community study where temperature and iron were elevated simultaneously (Rose et al. 2009). Temperature rather than iron had a stronger effect on ρ\textsubscript{NH4} and ρ\textsubscript{NO3} (Figure 2A, B). Results for ρ\textsubscript{PO4} by \textit{F. cylindrus} show that uptake of this substrate is affected by warming differently than the other inorganic substrates. Temperature elevation resulted in lower PO\textsubscript{4}\textsuperscript{3-} uptake rates (both absolute and per cell) compared to rates at 0°C (Figure 3A; Table 2). These results were reflected in uptake ratios, where both ρ\textsubscript{NO3}:ρ\textsubscript{PO4} and ρ\textsubscript{DIC}:ρ\textsubscript{PO4} significantly increased in the 4°C treatments (Figure 5; Table 3).

Compared to \textit{F. cylindrus}, the other pennate diatom in our study, \textit{P-n. subcurvata}, was affected by temperature and iron in a different manner. Within each temperature level, absolute uptake rates of all inorganic nutrient substrates were lower when Fe was present in high concentrations. This result was unexpected, and may be a function of the biomass that was present and the size of the cells. At both 0°C and 4°C, particulate concentrations (Figure S4) and cell abundances (Table 1) were lower when conditions were Fe replete. However, cell volumes (μm\textsuperscript{3}) and cellular C, N, and P quotas doubled when Fe concentrations were high compared to the respective low Fe treatments, resulting in lower surface area to volume ratios (results presented in Zhu et al. 2016). For \textit{P-n. subcurvata}, it is likely that iron addition results in larger cells with a higher cellular C, N, and P content, but the lower surface area to volume ratios may decrease their efficiency at taking up nutrients.

Regardless of the effect that high iron conditions had on cell size and physiology, our results show that warming increases absolute uptake rates of certain inorganic substrates by \textit{P-n. subcurvata}. When temperature alone was manipulated, ρ\textsubscript{DIC} and ρ\textsubscript{NO3}
significantly increased, and rates were significantly higher than those measured for *F. cylindrus* or *P. antarctica* (Figure 1A and 2B). The high $\rho_{\text{DIC}}$ and $\rho_{\text{NO}_3}$ measured in the 4C-Fe treatment could indicate that this species is physiologically inclined to grow well in iron limited environments, and that *Pseudo-nitzschia* spp. may have an advantage over other phytoplankton genera in a warmer Southern Ocean. The ability of the *Pseudo-nitzschia* genus to efficiently acquire iron when its concentration is low (Marchetti et al. 2006), as well as access intracellular iron pools more effectively at higher temperatures, has previously been postulated (Hutchins & Boyd 2016). Furthermore, an experiment looking at Southern Ocean microbial communities found that DIC and NO$_3^-$ uptake rates increase compared to ambient conditions when temperature alone is elevated (Spackeen et al. in prep a), and that *Pseudo-nitzschia* spp. becomes more prevalent than other phytoplankton groups (Xu et al. in prep). Results for both *P-n. subcurvata* and *F. cylindrus* in this study support the idea that a warmer Southern Ocean may favor diatoms over other phytoplankton groups (Boyd et al. 2016; Petrou et al. 2016). Our results also indicate that carbon fixation and new production could increase as a function of temperature even when Fe concentrations are low, which could enhance CO$_2$ drawdown and carbon export to the deep sea.

The relationship we observed between iron-replete conditions and absolute uptake of inorganic nutrients by *P-n. subcurvata* is somewhat counterintuitive and contrasts the conventional idea that nutrient utilization increases when Fe stress is removed. It is easier to interpret, however, when uptake rates are normalized to cell number. Uptake rates per cell in the iron-replete treatments for DIC, NO$_3^-$, and PO$_4^{3-}$ were higher than the iron-deplete treatments, and in some cases the difference was significant (Table 2). The
opposite pattern was observed for absolute uptake rates, indicating that bulk uptake rates can be uncoupled from cellular uptake rates. There is likely some point at which the advantages gained from increasing cell sizes are outweighed by the number of cells present. If we calculate absolute uptake rates of the iron-replete treatments and adjust for the difference in cell abundance by supposing that the iron-replete treatments had the same number of cells as the iron-deplete treatments, the adjusted absolute uptake rates in the iron-replete treatments become much greater than the iron-deplete treatments. As more research is completed on *Pseudo-nitzschia* spp., it is becoming increasingly evident that this genus has a versatile physiology and is able to employ a variety of strategies to help it thrive in different environments with varying nutrient regimes (Marchetti 2007; Boyd et al. 2016; Hutchins & Boyd 2016). Our results demonstrate that more research is needed to fully understand how iron affects *P-n. subcurvata* so that these impacts at the both the cellular level and community level can be placed into a global context to better predict how Southern Ocean phytoplankton communities and nutrient cycling may be different in the future.

Absolute uptake rates of inorganic nutrients by *P. antarctica* were affected by both temperature and iron; however, the impact of iron was greater than that of temperature as shown by consistently higher absolute uptake rates of all inorganic nutrients in the iron-replete treatments. Other studies have also observed a significant response of *P. antarctica* to iron addition with respect to growth rates (Alderkamp et al. 2012; Zhu et al. 2016). When temperature alone was elevated, $\rho_{DIC}$ and $\rho_{NO3}$ also significantly increased, however, the magnitude of increase was considerably less than that observed as a result of iron addition. Furthermore, uptake rates of all inorganic
substrates in the 4C-Fe treatment were significantly lower than the diatom species, indicating that *P. antarctica* may be at a competitive disadvantage in the Southern Ocean if warming occurs without a parallel increase in iron concentrations.

**Effect of Temperature and Iron on Organic Nutrient Uptake**

Compared to inorganic nutrient utilization data, measurements of organic nutrient uptake by Southern Ocean microorganisms are scarce, let alone how these rates are impacted by potential global change parameters. In this study, we identified a few trends for amino acid uptake that should be investigated further. Iron addition had a greater effect on uptake rates of amino acids than temperature elevation and the effect of iron on cellular uptake rates varied between the phytoplankton species (Table 2). For *P-n. subcurvata*, cellular uptake rates for both AA-C and AA-N significantly increased with iron addition, while decreases in cellular uptake rates for AA-N were measured in Fe replete treatments for *P. antarctica* at 0°C and at 4°C for *F. cylindrus*. Although it is likely that iron influences microbial utilization of amino acids, our measurements for absolute amino acid uptake had fewer significant results. The only occasion where absolute uptake rates of amino acids changed was when temperature and iron were elevated simultaneously resulting in significantly higher $\rho_{AA-C}$ and $\rho_{AA-N}$ by *P-n. subcurvata* (Figure 1B and 2D). Although little research has assessed the impacts of temperature and iron on the utilization of amino acids, a study of two different Ross Sea microbial communities found that absolute amino acid uptake rates increased when temperature and/or iron were elevated (Spackeen et al. in prep b). In the Southern Ocean, microbial utilization of amino acids is also influenced by irradiance (Rivkin & Putt 1987) and turnover rates are known to be high (Simon & Rosenstock 2007). The rate
measurements from this study demonstrate that utilization of amino acids is affected by iron availability; however, more research needs to be completed to fully understand the relationship. This particularly applies to *P-n. subcurvata*, where trends were significant for both cellular and absolute uptake rates. *Pseudo-nitzschia* spp. is a genus known to be able to use a variety of nitrogen substrates (Howard et al. 2007; Cochlan et al. 2008; Martin-Jézéquel 2015), and higher rates of amino acid utilization facilitated by increased iron concentrations could place this genus at a competitive advantage over other microorganisms who are not able to access amino acids as effectively.

Previous studies have found that urea uptake and the activity of urease, the enzyme involved in urea metabolism, increase under higher temperatures (Fan et al. 2003; Lomas & Glibert 1999; Spackeen et al. 2017). We did not find this to be the case for the phytoplankton species tested here (Figure 2C). Rather, iron addition had a larger effect on urea uptake, decreasing $\rho_{\text{UREA-N}}$ by *P-n. subcurvata* at both temperatures and increasing $\rho_{\text{UREA-N}}$ by *P. antarctica* at 0°C. These changes were similar to the patterns observed for inorganic nutrient uptake. For *P-n. subcurvata*, the same explanation described above with respect to cell volumes and reduced surface area to volume ratios could explain why $\rho_{\text{UREA-N}}$ decreased with iron addition. It is also possible that *P-n. subcurvata* preferentially utilizes more urea when iron is limiting as a way of decreasing its cellular iron requirement. Cells will use reduced forms of nitrogen, such as urea, when iron is limiting because oxidized forms of nitrogen require iron-containing enzymes (Marchetti et al. 2012). Iron availability and the urea cycle are tightly linked (Marchetti et al. 2012), and for diatoms, the expression of the urease enzyme can either vary (Milligan & Harrison 2000; Allen et al. 2011) or not change (Nunn et al. 2013) between
iron-replete and iron-deplete conditions. It is likely that iron addition impacts the utilization of urea by some species of phytoplankton and does not for others.

With respect to organic phosphorus, in the calculation for $\rho_{\text{ATP}}$, an assumed concentration of ATP was used; therefore, it is possible that the magnitude of the rates is inaccurate, but this should not affect the relative differences observed between the treatments and the phytoplankton species. Although phytoplankton use dissolved organic phosphorus, bacteria have historically been thought to assimilate it more readily (Björkman & Karl 1994). As a result, measurements of organic phosphorus uptake are uncommon, and the data that do exist are typically for marine heterotrophic bacteria or cyanobacteria (Fu et al. 2005; Casey et al. 2009; Michelou et al. 2011). Uptake of organic phosphorus is energetically more costly than inorganic phosphorus because phytoplankton are not able to take it up directly; rather, the $\text{PO}_4^{3-}$ group attached to the organic molecule becomes available through hydrolysis mediated by extracellular enzymes (Paytan & McLaughlin 2007 and references therein). Because of this, it is often thought that phytoplankton will only use organic phosphorus when $\text{PO}_4^{3-}$ is limiting. With respect to ATP, however, non-biological hydrolysis of the $\text{PO}_4^{3-}$ group in the $\gamma$ position can occur at typical seawater pH (Casey et al. 2009). In this study we used $\gamma$-AT$^{33}$P to determine uptake rates, so it is possible that both non-biological and biological processes mediated uptake of ATP.

In our study $\rho_{\text{ATP}}$ was used by all species, indicating that the phytoplankton that we tested use organic forms of phosphorus even when $\text{PO}_4^{3-}$ is available. Temperature and iron also significantly impacted $\rho_{\text{ATP}}$, thus, certain species of phytoplankton may be able to more effectively use ATP than others in the future. In particular, iron-replete
conditions resulted in significantly higher ATP uptake rates by both diatom species at 0°C, and temperature and iron synergistically increased \( \rho_{\text{ATP}} \) by \( P-n. \) subcurvata (Figure 3B).

**Effect of Temperature and Iron on Stoichiometric Ratios**

Temperature elevation and iron addition resulted in significant changes to both particulate ratios and the ratios at which nutrients were taken up (Figures 4 and 5; Table 3). Although stoichiometric changes were challenging to interpret, a few patterns stand out. C:P, N:P, and C:N ratios of Southern Ocean particulate matter typically fall below Redfield (Copin-Montegut & Copin-Montegut 1978; Martiny et al. 2013). The reason for the lower ratios is often attributed to the high abundance of diatoms (Kopczynska et al. 1986; Weber & Deutsch 2010), which usually have lower N:P ratios than other phytoplankton groups (Deutsch & Weber 2012; Planavsky 2014). We found that particulate N:P and C:P ratios of \( F. \) cylindrus and \( P-n. \) subcurvata were lower than \( P. \) antarctica and Redfield proportions (Figure 4). Higher temperature and iron resulted in significant changes to particulate ratios for the phytoplankton that we tested; however, the driver of change varied between the species. For \( F. \) cylindrus lower particulate C:N ratios and higher particulate N:P ratios occurred because concentrations of particulate nitrogen increased relatively more than particulate carbon and phosphorus. For \( P-n. \) subcurvata significant changes in particulate C:N were driven by particulate carbon concentrations, which decreased in the 0C+Fe treatment and increased in the higher temperature treatments. Patterns for \( P. \) antarctica were less clear, but a relative increase in particulate nitrogen concentrations resulted in lower C:N ratios in the high iron treatments, and a relative decrease in particulate phosphorus concentrations resulted in
higher C:P and N:P ratios in the 4C-Fe treatment. Our results support other studies that show that elemental ratios can significantly change when temperature and/or iron are manipulated (Doucette & Harrison 1991; Maldonado & Price 1996; De La Rocha et al. 2000; Dong et al. 2000; Berman-Frank et al. 2001; Price 2005; Xu et al. 2014); however, the direction of change is not consistent in these studies with some showing that C:N and N:P ratios increase while others show a decrease. Elemental ratios of primary producers span an order of magnitude and culture studies have demonstrated that species have remarkable flexibility (Geider & La Roche 2002; Weber & Deutsch 2010; Deutsch & Weber 2012; Planavsky 2014). Although more research needs to be conducted, our results indicate that particulate elemental ratios in the Southern Ocean may change in the future.

In this study, uptake ratios did not always have the same pattern as particulate ratios. *P.-n. subcurvata* was the only species where significant changes in uptake ratios were consistent with particulate ratios (Table 3). This may indicate that *P.-n. subcurvata* was able to more quickly adjust to the experimental conditions such that it was taking up nutrients in the appropriate ratios to match its cellular needs, while *F. cylindrus* and *P. antarctica* were still making physiological adjustments. Research has previously shown that uptake ratios of DIC and NO3- do not always reflect newly formed particulate organic matter (Collos et al. 1992; Banse 1994). Regardless of the inconsistencies between uptake ratios and particulate ratios, our data generally support the idea presented in Toseland et al. (2013) that at warmer temperatures, eukaryotic phytoplankton require fewer ribosomes to produce the required amounts of cellular proteins, which results in an increased nitrogen demand. As a result, C:P and N:P ratios increase as the rate of protein
synthesis increases and as the number of P-rich ribosomes decreases (Falkowski 2000; Geider & La Roche 2002). Although the trend was not observed in all of the 4°C treatments, in several cases, a significant increase in particulate ratios (C:P and N:P) and uptake ratios ($\rho_{\text{NO}_3}$:$\rho_{\text{PO}_4}$ and $\rho_{\text{DIC}}$:$\rho_{\text{PO}_4}$) occurred for both *F. cylindrus* and *P. antarctica* (Table 3). These results could indicate that the demand for N relative to P could increase in the future for these Southern Ocean species.

Stoichiometric results for *P-n. subcurvata* revealed a different story that was more centered on carbon. For both particulate ratios and uptake ratios, significant changes in the 4°C treatments were driven by a relative increase in particulate carbon and $\rho_{\text{DIC}}$ compared to nitrogen and phosphorus. These findings support the concept that higher temperatures can result in carbon overconsumption, which has been suggested in previous research on diatom cultures (Taucher et al. 2015) and in community studies where diatoms were dominant (Taucher et al. 2012; Spackeen et al. 2017). Carbon overconsumption by *P-n. subcurvata* and an increased demand for nitrogen observed for the other two species warrant further investigation because of the implications that they have for shaping the biogeochemistry of the Southern Ocean in the future.

*Future Significance for the Southern Ocean*

Here we present evidence that uptake rates and elemental stoichiometry of three important Southern Ocean phytoplankton species are impacted by elevated temperature and/or iron-replete conditions. Our study is unique in that we measured uptake rates of several carbon, nitrogen, and phosphorus substrates, including both inorganic and organic species. To our knowledge this is the first study to produce such a comprehensive set of uptake rate measurements for Southern Ocean phytoplankton species under current and
predicted future conditions. It is notable that the phytoplankton cultures used in our study had been recently isolated (i.e. ~16 months), adding to the environmental relevance of our data. Our results show that future Southern Ocean conditions affect the rates at which nutrients are taken up, and that the impact is not consistent between substrates. We also found that the three species that we tested had different responses to the experimental conditions, and that certain species may be able to take up selected substrates more readily than others depending on the situation. When iron is replete, both pennate diatoms and *P. antarctica* utilize most nutrients more quickly, although for *P-n. subcurvata* this trend is confounded by the complexities associated with absolute uptake rates versus cellular uptake rates. When temperature alone is elevated, our data indicate that pennate diatoms may be at an advantage over *P. antarctica*, and that *P-n subcurvata* may be particularly suited to thrive in a warmer Southern Ocean. Our data also provide evidence that nutrient ratios will likely change in the future due to a higher nitrogen demand for some species and carbon overconsumption for others. Although uncertainties remain, this study highlights the importance of studying different species and multiple substrates in order to gain a broader understanding of how the community structure and nutrient cycling of the Southern Ocean may be different in the future.
Acknowledgements: The authors would like to acknowledge M.P. Sanderson and Q.N. Roberts for their assistance with analyzing samples, and E.M. Bertrand and F-X. Fu for their help running the experiment. Thanks to A.O. Tatters and J.B. McQuaid for isolating the phytoplankton strains used in this experiment. This research was supported by grant numbers 1043635 and 1043748, awarded to D.A. Bronk and D.A. Hutchins respectively from the National Science Foundation. This paper is Contribution No. XXX of the Virginia Institute of Marine Science, College of William & Mary.
LITERATURE CITED


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dynamics of size structured photosynthesis parameters (PAM) and primary production \(^{13}\text{C}\) of pico- and nano-phytoplankton in an atoll lagoon. Mar Pollut Bull 65:478-489


Table 1) Chlorophyll a (Chl a) concentrations, cell counts, and nutrient concentrations of the two diluent types (Diluent -Fe and Diluent +Fe) and the different treatments for each phytoplankton species. Data are presented as the mean ± 1 standard deviation (n=3). DON includes both urea and DPA. Cell counts for *Phaeocystis antarctica* in the 4°C treatments were excluded due to poor preservation. For each phytoplankton species, Chl a concentrations and cell abundances that have a * next to them indicate that a treatment is significantly different (p < 0.05) from the respective 0C-Fe control.

<table>
<thead>
<tr>
<th>Source</th>
<th>Chl a µg L⁻¹</th>
<th>Algal Cells 10⁴ cells mL⁻¹</th>
<th>DOC µmol C L⁻¹</th>
<th>NH₄⁺ µmol N L⁻¹</th>
<th>NO₃⁻ µmol N L⁻¹</th>
<th>DON µmol N L⁻¹</th>
<th>Urea µmol N L⁻¹</th>
<th>DPA µmol N L⁻¹</th>
<th>PO₄³⁻ µmol P L⁻¹</th>
<th>Si µmol Si L⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diluent -Fe</td>
<td>N/A</td>
<td>N/A</td>
<td>596 ± 8.6</td>
<td>1.70 ± 0.5</td>
<td>53.5 ± 0.2</td>
<td>86.1 ± 0.3</td>
<td>0.70 ± 0.0</td>
<td>0.30 ± 0.3</td>
<td>5.46 ± 0.1</td>
<td>68.0 ± 0.0</td>
</tr>
<tr>
<td>Diluent +Fe</td>
<td>N/A</td>
<td>N/A</td>
<td>620 ± 48</td>
<td>2.18 ± 0.0</td>
<td>50.0 ± 0.0</td>
<td>91.9 ± 1.1</td>
<td>0.77 ± 0.0</td>
<td>0.07 ± 0.0</td>
<td>5.18 ± 0.1</td>
<td>68.0 ± 0.0</td>
</tr>
<tr>
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<tr>
<td>0C-Fe</td>
<td>2.87 ± 0.8</td>
<td>8.56 ± 0.9</td>
<td>566 ± 3.6</td>
<td>0.98 ± 0.1</td>
<td>48.4 ± 0.4</td>
<td>89.0 ± 0.8</td>
<td>0.28 ± 0.0</td>
<td>0.10 ± 0.0</td>
<td>4.52 ± 0.0</td>
<td>61.1 ± 0.4</td>
</tr>
<tr>
<td>0C+Fe</td>
<td>12.4 ± 0.6 *</td>
<td>11.0 ± 0.8</td>
<td>575 ± 2.7</td>
<td>1.67 ± 1.0</td>
<td>36.3 ± 1.0</td>
<td>91.6 ± 1.6</td>
<td>0.31 ± 0.0</td>
<td>0.13 ± 0.0</td>
<td>3.81 ± 0.1</td>
<td>55.0 ± 0.8</td>
</tr>
<tr>
<td>4C-Fe</td>
<td>5.63 ± 0.2</td>
<td>12.0 ± 0.6</td>
<td>563 ± 4.7</td>
<td>0.93 ± 0.1</td>
<td>47.4 ± 0.3</td>
<td>88.1 ± 1.8</td>
<td>0.18 ± 0.0</td>
<td>0.08 ± 0.0</td>
<td>4.41 ± 0.0</td>
<td>58.2 ± 0.4</td>
</tr>
<tr>
<td>4C+Fe</td>
<td>15.9 ± 2.8 *</td>
<td>22.9 ± 2.5 *</td>
<td>572 ± 8.1</td>
<td>1.11 ± 0.0</td>
<td>33.0 ± 2.2</td>
<td>90.4 ± 0.0</td>
<td>0.20 ± 0.1</td>
<td>0.10 ± 0.0</td>
<td>3.54 ± 0.2</td>
<td>49.7 ± 2.0</td>
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<tr>
<td><strong>Pseudo-nitzschia subcurvata</strong></td>
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<tr>
<td>0C-Fe</td>
<td>16.1 ± 0.6</td>
<td>7.19 ± 0.4</td>
<td>534 ± 11</td>
<td>0.94 ± 0.1</td>
<td>40.7 ± 0.6</td>
<td>84.4 ± 1.5</td>
<td>0.19 ± 0.0</td>
<td>0.10 ± 0.0</td>
<td>3.69 ± 0.4</td>
<td>48.2 ± 6.5</td>
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<td>0C+Fe</td>
<td>13.0 ± 1.1</td>
<td>1.91 ± 0.2 *</td>
<td>579 ± 1.2</td>
<td>1.25 ± 0.2</td>
<td>40.9 ± 0.5</td>
<td>92.4 ± 1.2</td>
<td>0.54 ± 0.0</td>
<td>0.10 ± 0.0</td>
<td>4.07 ± 0.6</td>
<td>53.2 ± 8.8</td>
</tr>
<tr>
<td>4C-Fe</td>
<td>21.0 ± 0.9 *</td>
<td>9.11 ± 1.5</td>
<td>546 ± 1.1</td>
<td>1.03 ± 0.1</td>
<td>37.7 ± 0.4</td>
<td>85.0 ± 1.0</td>
<td>0.26 ± 0.1</td>
<td>0.13 ± 0.1</td>
<td>3.54 ± 0.4</td>
<td>43.8 ± 5.4</td>
</tr>
<tr>
<td>4C+Fe</td>
<td>24.8 ± 3.5 *</td>
<td>3.85 ± 0.2 *</td>
<td>564 ± 3.9</td>
<td>1.21 ± 0.1</td>
<td>34.1 ± 1.5</td>
<td>85.5 ± 0.7</td>
<td>0.33 ± 0.1</td>
<td>0.13 ± 0.0</td>
<td>3.83 ± 0.1</td>
<td>55.5 ± 1.0</td>
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<td><strong>Phaeocystis antarctica</strong></td>
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<tr>
<td>0C-Fe</td>
<td>1.40 ± 0.2</td>
<td>1.72 ± 0.3</td>
<td>553 ± 5.8</td>
<td>0.80 ± 0.1</td>
<td>48.8 ± 0.1</td>
<td>87.9 ± 0.2</td>
<td>0.63 ± 0.0</td>
<td>0.10 ± 0.0</td>
<td>4.76 ± 0.0</td>
<td>68.7 ± 0.2</td>
</tr>
<tr>
<td>0C+Fe</td>
<td>4.23 ± 0.2 *</td>
<td>4.38 ± 0.4 *</td>
<td>533 ± 5.8</td>
<td>0.83 ± 0.1</td>
<td>32.4 ± 1.5</td>
<td>85.1 ± 0.3</td>
<td>0.43 ± 0.0</td>
<td>0.13 ± 0.0</td>
<td>3.84 ± 0.1</td>
<td>68.8 ± 0.1</td>
</tr>
<tr>
<td>4C-Fe</td>
<td>2.05 ± 0.4</td>
<td>539 ± 4.0</td>
<td>0.67 ± 0.0</td>
<td>49.3 ± 0.1</td>
<td>85.9 ± 0.7</td>
<td>0.51 ± 0.0</td>
<td>0.08 ± 0.0</td>
<td>4.82 ± 0.0</td>
<td>68.2 ± 0.0</td>
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<tr>
<td>4C+Fe</td>
<td>5.86 ± 0.3 *</td>
<td>531 ± 7.5</td>
<td>0.91 ± 0.1</td>
<td>34.0 ± 0.4</td>
<td>84.3 ± 1.1</td>
<td>0.51 ± 0.0</td>
<td>0.09 ± 0.0</td>
<td>4.08 ± 0.0</td>
<td>67.8 ± 0.1</td>
<td></td>
</tr>
</tbody>
</table>
Table 2) Mean uptake rates per cell of the different carbon, nitrogen, and phosphorus substrates by *Fragilariopsis cylindrus*, *Pseudo-nitzschia subcurvata*, and *Phaeocystis antarctica*. Rates are expressed as fmol of either C, N, or P cell\(^{-1}\) h\(^{-1}\), with the exception of ATP, which is expressed as amol P cell\(^{-1}\) h\(^{-1}\). Data are presented as the mean ± 1 SD (n=3). For each phytoplankton species, values that have a * next to them indicate that a treatment is significantly different (p < 0.05) from the respective 0C-Fe control. Uptake rates per cell were not determined (ND) for the *Phaeocystis antarctica* 4°C treatments due to poor preservation of the cells.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>DIC fmol C cell(^{-1}) h(^{-1})</th>
<th>AA-C fmol C cell(^{-1}) h(^{-1})</th>
<th>NH(_4^+) fmol N cell(^{-1}) h(^{-1})</th>
<th>NO(_3^-) fmol N cell(^{-1}) h(^{-1})</th>
<th>Urea fmol N cell(^{-1}) h(^{-1})</th>
<th>AA-N fmol N cell(^{-1}) h(^{-1})</th>
<th>PO(_4^{3-}) fmol P cell(^{-1}) h(^{-1})</th>
<th>ATP amol P cell(^{-1}) h(^{-1})</th>
</tr>
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<tbody>
<tr>
<td><strong>Fragilariopsis cylindrus</strong></td>
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<tr>
<td>0C-Fe</td>
<td>5.32 ± 0.5</td>
<td>0.64 ± 0.2</td>
<td>1.53 ± 0.7</td>
<td>1.86 ± 0.1</td>
<td>0.59 ± 0.3</td>
<td>0.28 ± 0.1</td>
<td>0.27 ± 0.1</td>
<td>0.11 ± 0.0</td>
</tr>
<tr>
<td>0C+Fe</td>
<td>8.38 ± 0.7 *</td>
<td>0.47 ± 0.1</td>
<td>1.26 ± 0.5</td>
<td>2.04 ± 0.1</td>
<td>0.34 ± 0.0</td>
<td>0.23 ± 0.0</td>
<td>0.28 ± 0.1</td>
<td>0.22 ± 0.0 *</td>
</tr>
<tr>
<td>4C-Fe</td>
<td>7.20 ± 0.6 *</td>
<td>0.53 ± 0.0</td>
<td>2.08 ± 0.3</td>
<td>2.33 ± 0.3</td>
<td>0.43 ± 0.0</td>
<td>0.20 ± 0.0</td>
<td>0.11 ± 0.0</td>
<td>0.05 ± 0.0 *</td>
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<tr>
<td>4C+Fe</td>
<td>6.16 ± 0.5</td>
<td>0.22 ± 0.1</td>
<td>0.70 ± 0.1 *</td>
<td>1.28 ± 0.0</td>
<td>0.25 ± 0.1</td>
<td>0.12 ± 0.0 *</td>
<td>0.06 ± 0.0 *</td>
<td>0.06 ± 0.0 *</td>
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<td><strong>Pseudo-nitzschia subcurvata</strong></td>
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<td>0C-Fe</td>
<td>17.2 ± 0.6</td>
<td>0.50 ± 0.1</td>
<td>3.29 ± 0.5</td>
<td>4.44 ± 0.2</td>
<td>0.68 ± 0.1</td>
<td>0.21 ± 0.0</td>
<td>0.19 ± 0.1</td>
<td>0.10 ± 0.1</td>
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<tr>
<td>0C+Fe</td>
<td>19.2 ± 2.0</td>
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<td>3.01 ± 0.5</td>
<td>6.42 ± 0.7</td>
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<td>0.79 ± 0.4 *</td>
<td>0.36 ± 0.0</td>
<td>0.87 ± 0.1 *</td>
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<td>4C-Fe</td>
<td>30.3 ± 6.7 *</td>
<td>0.81 ± 0.2</td>
<td>2.86 ± 0.3</td>
<td>5.15 ± 1.1</td>
<td>0.76 ± 0.2</td>
<td>0.26 ± 0.1</td>
<td>0.25 ± 0.1</td>
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<tr>
<td>4C+Fe</td>
<td>41.5 ± 0.5 *</td>
<td>2.45 ± 0.3 *</td>
<td>2.63 ± 0.2</td>
<td>6.38 ± 0.7</td>
<td>0.40 ± 0.2</td>
<td>0.84 ± 0.1 *</td>
<td>0.35 ± 0.0</td>
<td>0.79 ± 0.1 *</td>
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<tr>
<td><strong>Phaeocystis antarctica</strong></td>
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<tr>
<td>0C-Fe</td>
<td>7.30 ± 1.6</td>
<td>3.09 ± 0.6</td>
<td>6.34 ± 0.4</td>
<td>5.42 ± 1.2</td>
<td>0.35 ± 0.2</td>
<td>1.49 ± 0.4</td>
<td>0.30 ± 0.1</td>
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<td>0C+Fe</td>
<td>29.1 ± 2.8</td>
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<td>4.42 ± 1.0</td>
<td>6.78 ± 1.0</td>
<td>0.28 ± 0.0</td>
<td>0.51 ± 0.1 *</td>
<td>0.25 ± 0.0</td>
<td>0.24 ± 0.0 *</td>
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<td>ND</td>
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<tr>
<td>4C+Fe</td>
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</table>

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Table 3) Particulate ratios (C:P, N:P, C:N) and uptake ratios ($\rho_{\text{DIC}}:\rho_{\text{PO}_4}$, $\rho_{\text{NO}_3}:\rho_{\text{PO}_4}$, $\rho_{\text{DIC}}:\rho_{\text{NO}_3}$) that changed significantly (p < 0.05) compared to the 0C-Fe control for each phytoplankton species. Arrows indicate whether a ratio significantly increased or decreased.

<table>
<thead>
<tr>
<th>Phytoplankton Species</th>
<th>C:P</th>
<th>N:P</th>
<th>C:N</th>
<th>$\rho_{\text{DIC}}:\rho_{\text{PO}_4}$</th>
<th>$\rho_{\text{NO}<em>3}:\rho</em>{\text{PO}_4}$</th>
<th>$\rho_{\text{DIC}}:\rho_{\text{NO}_3}$</th>
</tr>
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<tr>
<td>0C+Fe</td>
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<tr>
<td>4C-Fe</td>
<td>↓</td>
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<tr>
<td>4C+Fe</td>
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<tr>
<td><em>Pseudo-nitzschia subcurvata</em></td>
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<tr>
<td>0C+Fe</td>
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<td>↓</td>
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<tr>
<td>4C-Fe</td>
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<td><em>Phaeocystis antarctica</em></td>
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<td>4C-Fe</td>
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Figure 1) Mean absolute carbon uptake rates of (A) DIC and (B) amino acid carbon (AA-C) by *Fragilariopsis cylindrus* (black bars), *Pseudo-nitzschia subcurvata* (white bars), and *Phaeocystis antarctica* (gray bars). Rates are expressed as µmol C L⁻¹ h⁻¹. Data are presented as the mean ± 1 SD (n=3). Within each plot, bars that have different letters are significantly different from one another (p < 0.05).
Figure 2) Mean absolute nitrogen uptake rates of (A) NH$_4^+$ (B) NO$_3^-$ (C) urea, and (D) amino acid nitrogen (AA-N) by Fragilariopsis cylindrus (black bars), Pseudo-nitzschia subcurvata (white bars), and Phaeocystis antarctica (gray bars). Rates are expressed as µmol N L$^{-1}$ h$^{-1}$. Data are presented as the mean ± 1 SD (n=3). Within each plot, bars that have different letters are significantly different from one another (p < 0.05).
Figure 3) Mean absolute phosphorus uptake rates of (A) PO$_4^{3-}$ and (B) ATP by Fragilariopsis cylindrus (black bars), Pseudo-nitzschia subcurvata (white bars), and Phaeocystis antarctica (gray bars). Rates for PO$_4^{3-}$ are expressed as µmol P L$^{-1}$ h$^{-1}$ and rates for ATP are expressed as nmol P L$^{-1}$ h$^{-1}$. Within each plot, data are presented as the mean ± 1 SD (n=3). Bars that have different letters are significantly different from one another (p < 0.05).
Figure 4) Mean cellular particulate ratios for *Fragilariopsis cylindrus* (black circles), *Pseudo-nitzschia subcurvata* (white squares), and *Phaeocystis antarctica* (gray triangles) at 0C-Fe (A), 0C+Fe (B), 4C-Fe (C), and 4C+Fe (D). Redfield stoichiometry is shown as a reference (×=106:16:1; dotted line has a slope of 6.6, which is Redfield C:N; points above the dotted line have C:N < 6.6 and points below have C:N > 6.6). Data are presented as the mean ± 1 SD (n=3).
Figure 5) Mean uptake ratios for *Fragilariopsis cylindrus* (black circles), *Pseudo-nitzschia subcurvata* (white squares), and *Phaeocystis antarctica* (gray triangles) at 0C-Fe (A), 0C+Fe (B), 4C-Fe (C), and 4C+Fe (D). Redfield stoichiometry is shown as a reference (×=106:16:1; dotted line has a slope of 6.6, which is Redfield C:N; points above the dotted line have $\frac{\rho_{\text{DIC}}}{\rho_{\text{NO3}}} < 6.6$ and points below have $\frac{\rho_{\text{DIC}}}{\rho_{\text{NO3}}} > 6.6$). Data are presented as the mean ± 1 SD (n=3).
Supplemental Materials

**Figure S1** Mean specific carbon uptake rates of (A) DIC and (B) amino acid carbon (AA-C) by *Fragilariopsis cylindrus* (black bars), *Pseudo-nitzschia subcurvata* (white bars), and *Phaeocystis antarctica* (gray bars). Rates are expressed as h⁻¹. Data are presented as the mean ± 1 SD (n=3). Within each plot, bars that have different letters are significantly different from one another (p < 0.05).
Figure S2) Mean specific nitrogen uptake rates of (A) NH$_4^+$ (B) NO$_3^-$ (C) urea, and (D) amino acid nitrogen (AA-N) by Fragilariopsis cylindrus (black bars), Pseudo-nitzschia subcurvata (white bars), and Phaeocystis antarctica (gray bars). Rates are expressed as h$^{-1}$. Data are presented as the mean ± 1 SD (n=3). Within each plot, bars that have different letters are significantly different from one another (p < 0.05).
Figure S3) Mean specific phosphorus uptake rates of (A) PO$_4^{3-}$ and (B) ATP by *Fragilariopsis cylindrus* (black bars), *Pseudo-nitzschia subcurvata* (white bars), and *Phaeocystis antarctica* (gray bars). Rates are expressed as h$^{-1}$. Within each plot, data are presented as the mean ± 1 SD (n=3). Bars that have different letters are significantly different from one another (p < 0.05).
Figure S4) Mean particulate concentrations of (A) particulate carbon, (B) particulate nitrogen, and (C) particulate phosphorus for *Fragilariopsis cylindrus* (black bars), *Pseudo-nitzschia subcurvata* (white bars), and *Phaeocystis antarctica* (gray bars). Concentrations are expressed as µmol C, N, or P L⁻¹. Within each plot, data are presented as the mean ± 1 SD (n=3). Bars that have different letters are significantly different from one another (p < 0.05).
CHAPTER 6

SUMMARY AND CONCLUDING REMARKS
A variety of experimental approaches were used in this dissertation to investigate the microbial response to simulated future conditions that will likely develop over the next century. The major goal of this research was to determine how changing environmental conditions (e.g., temperature, CO$_2$, and nutrient sources) impact the rates at which microorganisms use different carbon, nitrogen, and phosphorus substrates. This work is unique because fully factorial experimental designs were used so that the individual and combined affects of multiple parameters could be assessed. At both the Southern California Bight and Ross Sea study sites, the impacts of elevated temperature and higher CO$_2$ were studied. Additionally, the dominant nitrogen substrate present in the seawater was another variable looked at in the Southern California Bight, and iron addition and changing N:P supply ratios were examined in the Ross Sea. Collectively the data show that nutrient cycles will likely be different in the future.

**Key Findings**

Temperature was one of the main variables tested in this dissertation research. A temperature increase of 4°C was investigated in all chapters, and the data show that temperature elevation increases nutrient uptake rates. In both the Southern California Bight and the Ross Sea, uptake rates of dissolved inorganic carbon (DIC) significantly increased as a function of temperature. Nitrogen uptake rates were also affected by elevated temperatures— urea uptake significantly increased in the Southern California Bight (Chapter 2) and nitrate (NO$_3^-$) uptake rates significantly increased in the Ross Sea for larger microorganisms (Chapter 3) and for smaller microorganisms (Chapter 4). In studies where diatoms were prevalent (Chapters 2 and 3), temperature had a larger impact...
than the other parameters that were manipulated. When individual Southern Ocean diatom species (*Fragilariopsis cylindrus* and *Pseudo-nitzschia subcurvata*) were tested, temperature elevation also increased DIC and NO\textsubscript{3} uptake rates (Chapter 5). When *Phaeocystis antarctica* was the dominant species in the community (Chapter 4) and when *Phaeocystis antarctica* was studied in culture (Chapter 5), temperature elevation had less of an impact on nutrient uptake rates, however, temperature was still identified as the variable that exerted the strongest influence on community composition (Chapter 4).

These results indicate that a warmer Southern Ocean may favor diatoms over other phytoplankton groups like *Phaeocystis* spp. These findings also support other studies that show that Southern Ocean diatom physiology is strongly influenced by temperature and that diatoms may be at a competitive advantage in the future (Boyd et al. 2016; Petrou et al. 2016; Zhu et al. 2016).

My results also show that increasing CO\textsubscript{2} from current levels (380-400 ppm) to 800 ppm did not considerably impact nutrient uptake rates (Chapters 2 and 3). In both the Southern California Bight and the Ross Sea, uptake rates either did not significantly change when CO\textsubscript{2} was elevated or changes could be attributed to other parameters that were manipulated (i.e. temperature or Fe). The only exception was that NO\textsubscript{3} uptake rates by smaller microorganisms in the Southern California Bight significantly increased, however, this relationship may only be relevant if elevations in temperature and CO\textsubscript{2} are decoupled in the future, as NO\textsubscript{3} uptake rates were not impacted when CO\textsubscript{2} and temperature were increased in combination. Studies find that nitrogen utilization can increase when CO\textsubscript{2} is elevated (Beardall et al. 2009) or be unaffected (Engel et al. 2005; Riebesell et al. 2007). The Southern California Bight and the Ross Sea are two locations
characterized by higher CO$_2$ conditions relative to other oceanic regions, so it is possible that the microorganisms at these sites have physiology that is already optimized for high CO$_2$ conditions (Hauri et al. 2009). Previous research suggests that photosynthesis of Southern Ocean microorganisms is already nearly CO$_2$-saturated from diffusion (Gleitz et al. 1996; Raven et al. 2002; Kranz et al. 2015), and that diatoms in general may be less susceptible to high CO$_2$ conditions compared to other phytoplankton groups because they have efficient carbon concentrating mechanisms (Tortell et al. 2002; Fabry et al. 2008).

Iron addition was studied in all experiments that occurred in the Ross Sea (Chapters 3-5). Iron typically limits phytoplankton growth in the Southern Ocean (Martin 1990), so it was expected that iron addition would increase biomass and nutrient uptake rates. Studies from this dissertation indicate that iron addition generally increases biomass and nutrient uptake rates, however, results vary depending on which phytoplankton groups are dominant. When diatoms were dominant, iron addition at 0°C increased biomass, however, the effects of iron addition were more apparent at 4°C and an additive effect of temperature elevation and iron addition was observed for DIC and NO$_3^-$ uptake rates (Chapter 3). This result supports the idea that iron addition boosts cellular pathways that require iron, and that diatoms can access cellular iron pools more effectively when temperature is increased (Hutchins & Boyd 2016). It also provides evidence that both the cold-water temperature and the low concentrations of iron in the Southern Ocean may limit diatom growth (Reay et al. 2001). When *Phaeocystis antarctica* was dominant, iron addition resulted in significantly higher DIC and NO$_3^-$ uptake rates at both 0°C and 4°C, and impacts from temperature only occurred in the smaller size fraction where *Phaeocystis antarctica* was not present (Chapter 4). When
*Phaeocystis antarctica* was studied in culture, iron addition, rather than temperature elevation, also resulted in the highest uptake rates of inorganic nutrients (Chapter 5). These results support other studies that demonstrate that *Phaeocystis antarctica* growth rates significantly increase with iron addition and that *Phaeocystis antarctica* may be more sensitive to temperature change than other Southern Ocean phytoplankton groups (Alderkamp et al. 2012; Xu et al. 2014; Zhu et al. 2016).

Stoichiometric nutrient utilization, cellular C:N:P elemental ratios, and changing the dissolved N:P supply ratio were additional concepts that were studied in this dissertation. When temperature was increased, carbon overconsumption was observed for Southern California Bight microorganisms (Chapter 2) and for *Pseudo-nitzschia subcurvata* (Chapter 5), which supports research showing that diatoms may use relatively more carbon at higher temperatures (Taucher et al. 2012; Taucher et al. 2015). Carbon overconsumption did not occur for *Fragilariopsis cylindrus* and *Phaeocystis antarctica* at higher temperatures, rather, stoichiometric uptake ratios and cellular particulate ratios reflected an increased demand for nitrogen and less of a need for phosphorus (Chapter 5). These results fall in line with the idea that at higher temperatures, fewer P-rich ribosomes are needed to produce the required amount of N-rich cellular proteins (Toseland et al. 2013). Two experiments from this dissertation investigated how Ross Sea microorganisms respond when the dissolved N:P ratio of the seawater is decreased and increased compared to ambient conditions (Chapter 4). The data from these experiments suggest that changes to dissolved N:P ratios can impact microbial growth and nutrient uptake over short times scales (i.e. a few days) and result in subtle shifts to the composition of the phytoplankton community, but that other global change parameters
(i.e., temperature and iron) have a stronger impact on microbial nutrient utilization and community composition.

DIC and NO$_3^-$ uptake rates were a large focus of the research in this dissertation and were measured in all experiments; however, uptake rates of a number of other nutrient substrates were also analyzed. Community uptake rates of urea by Southern California Bight microorganisms (Chapter 2) and amino acids by Ross Sea microorganisms (Chapters 4) were measured in addition to DIC and NO$_3^-$ uptake. Results from these experiments demonstrate that microbial utilization of organic nutrients can be higher than utilization of inorganic substrates, and that global change parameters have the potential to influence uptake rates of organic substrates. Urea uptake rates were greater than NO$_3^-$ and increased significantly when temperature was increased (Chapter 2). Amino acid-N uptake rates were greater than NO$_3^-$ uptake rates for smaller microorganisms during one of the experiments presented in Chapter 4, and amino acid uptake typically increased when iron was added. Uptake rates of several inorganic and organic carbon, nitrogen, and phosphorus substrates by Southern Ocean phytoplankton species were measured in Chapter 5, producing the most inclusive set of uptake rate measurements for Southern Ocean phytoplankton species under current and predicted future conditions to date.

**Synopsis and Future Directions**

The research presented in this dissertation provides a comprehensive view of how microbial organisms in both temperate and polar regions may respond to global change parameters and how nutrient cycles may be impacted in the future. The factorial design
of the experiments allowed the effects of tested variables to be interpreted individually as well as in combination with other parameters. This made it possible to identify which variable(s) will likely be the largest driver(s) of change over the next century. Although all variables tested were found to exert some influence on microbial nutrient cycling, temperature elevation stood out as being the parameter that resulted in the largest amount of change, significantly increasing biomass and uptake rates, structuring the composition of the microbial community, and altering stoichiometry. Iron was also found to be an important driver of change in the Ross Sea, particularly when *Phaeocystis antarctica* was prominent or when it was manipulated in combination with temperature. Increasing CO$_2$ and changing the dissolved N:P supply ratio had less of an impact than the other variables tested.

This research has added to the body of knowledge on temperate and polar biogeochemistry, and it has provided a robust data set on how uptake rates are impacted by multiple global change parameters; however, there are several avenues for future research that could build upon the findings described here. In the studies presented here, only two levels of temperature, CO$_2$, and iron were examined. While this design provided information regarding how nutrient cycling may be different within the next century, it is limited in being able to predict further into the future. Designing the same experiments but adding more levels would allow curves to be plotted such that optimum physiological levels could be identified. It would be particularly useful to test several temperature levels, as phytoplankton species have varying optimum temperatures for growth (Thomas et al. 2012). It would also be interesting to repeat these studies and focus on NH$_4^+$, as it is often the preferred nitrogen substrate for phytoplankton nitrogen
uptake (McCarthy 1981; Glibert et al. 2016 and references therein). Along these same lines, it would be worthwhile to isolate other important phytoplankton species, and conduct more studies in culture to further understand how global change parameters affect nutrient uptake rates. Lastly, the research in this dissertation is limited temporally and spatially, thus, our findings would be more robust if future studies could be conducted at different stages of the phytoplankton bloom and at more sites within the coastal California region and the Southern Ocean.
LITERATURE CITED


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community composition, with emphasis on nitrogen-enriched conditions.

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