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Seasonal Nitrogen Uptake and Regeneration in the Water Column and Sea-Ice of the Western Coastal Arctic

Steven E. Baer

College of William and Mary - Virginia Institute of Marine Science

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Seasonal Nitrogen Uptake and Regeneration in the Water Column and Sea-Ice of the
Western Coastal Arctic

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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

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by
Steven E. Baer

2013
APPROVAL SHEET

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

Doctor of Philosophy

Steven E. Baer

Approved July 29, 2013

Deborah A. Brock, Ph.D.
Committee Chairman/Advisor

Deborah K. Steinberg, Ph.D.

Marjorie A. M. Friedrichs, Ph.D.

Michael W. Lomas, Ph.D.
Bigelow Laboratory for Ocean Science
East Boothbay, ME

David L. Kirchman, Ph.D.
University of Delaware
Lewes, DE
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Abstract

The logistical difficulties of research in extremely low temperatures and lack of access to the Arctic have meant that there is a historic dearth of knowledge of coastal Arctic biogeochemistry, especially during winter when sea ice is present. Recent observations, however, indicate that the Arctic is changing rapidly. Changes include increased temperatures, decreased extent and volume of sea ice, and increased freshwater inputs. How these changes influence biogeochemical cycles is an open question, especially in the highly productive coastal regions of the Chukchi Sea. Here I present nitrogen (N) uptake and regeneration rates for phytoplankton and bacteria measured in the shallow waters and landfast sea ice near Barrow, Alaska. Experiments were performed using tracer-level incubations of stable isotope ($^{15}$N) ammonium, nitrate, urea, and amino acids during January, April, and August over two successive years (2010 – 2012). Autotrophic versus heterotrophic N uptake was measured with traditional size fractionation. In brief, I found that outside of the spring phytoplankton bloom period, ammonium and amino acids were the preferred N substrates assimilated. Regeneration of N and nitrification were especially high during winter. A high-speed cell sorting flow cytometer was used to distinguish bacterial sub-populations and their uptake rates. Low nucleic acid populations were active in taking up N compounds, although not at quite the same rate as high nucleic acid cells. The difference was less pronounced during winter compared to summer. Additional experiments were designed to artificially warm the samples to demonstrate that ammonium uptake rates increased with temperature and substrate availability, whereas nitrification rates did not. Uptake and regeneration of ammonium and nitrate along with nitrification was also measured in landfast sea ice. This is the first report of N uptake from within the sea ice matrix in the Chukchi Sea. Given the paucity of information on N cycling in the Arctic Ocean, these data can inform modeling efforts to predict future changes in the system and also provide a baseline by which to compare future observations.
Nitrogen Uptake in the Western Coastal Arctic
Chapter 1 – Introduction
The Arctic Ocean has relatively wide continental shelves, substantial freshwater flux from rivers, and significant Pacific Ocean inputs, such that it resembles a large estuary rather than an oceanic basin (Carmack and Wassmann 2006; Dittmar and Kattner 2003; McClelland et al. 2012). Compared to the size of the basin, the Arctic receives extraordinarily large freshwater inputs from rivers and continental runoff relative to the other oceans (Aagaard and Carmack 1989). In the western Arctic, the Chukchi Sea also receives relatively low salinity Pacific water through the Bering Strait (Roach et al. 1995). Surface waters tend to flow northeast past Barrow, Alaska and out into the Beaufort Gyre. In addition to input fluxes from surrounding seas and terrestrial areas, sea ice within the system adds salt during formation and releases fresh water during melt (Carmack 2007). Organisms in the Arctic must tolerate rapid changes in salinity, temperature, and light caused by seasonal changes in sea ice cover. Despite those apparent challenges, secondary production in the Chukchi Sea is a high (Bates et al. 2005; Sakshaug 2004).

Of pressing concern to Arctic science is how the system will react to climate change. Since there is relatively little data on the biogeochemistry of the Arctic, it is difficult to predict the consequences of changes in the marine ecosystem of the region. The goals of this project were to provide a current empirical baseline of N cycling processes along the coastal Chukchi Sea during winter, spring and summer. Field sampling for this dissertation was based out of the former Naval Arctic Research Station in Barrow, Alaska. New facilities located there allowed us access to the landfast sea ice and coastal region during year-round seasonal sampling, along with modern laboratory facilities and walk-in incubators. Experiments performed in the water column and sea ice
provide a unique set of data that shows the importance of regenerated and organic N sources to the coastal Chukchi ecosystem along with information on how these processes may change with future increases in temperature. By way of introduction to the dissertation, below I briefly review information about nutrients and nitrogen cycling, the climate changes occurring, and the unique environment of sea ice.

**Nutrients and nitrogen cycling**

The Pacific Ocean delivers surface waters with high concentrations of nutrients essential for biological growth, such as nitrate, phosphate, and silica (Codispoti et al. 2005; Codispoti et al. 2009). These nutrients along with those supplied by sea ice retreat (Carmack and Chapman 2003) support intense seasonal pulses of productivity in the Chukchi Sea (Dyda et al. 2009). The spring thaw results in the transport of riverine dissolved organic matter (DOM) to the coastal ocean, which can provide labile material that is likely important to both primary and secondary production (Kirchman et al. 2009; Wickland et al. 2012).

Light and community composition control the timing and fate of the spring production pulse, but the primary control on annual net community production is nitrogen supply (Tremblay et al. 2006; Walsh et al. 2004). Nitrogen plays a central role in ocean biogeochemistry because it is a constituent of amino acids, proteins and nucleic acids, and is therefore fundamental to all living things. Marine organisms mediate transformations among different N forms as part of their metabolism, either for growth or energy. As such, the availability, uptake, and remineralization of N are important
controls on biomass production, and N is subject to fierce competition by autotrophic and heterotrophic organisms. This balance between autotrophs and heterotrophs is a fundamental driver of important ecological parameters in the marine environment and has important implications for food web dynamics and carbon (C) exchange with the atmosphere.

Despite the importance of N to Arctic microorganisms, there are limited studies of N uptake rates in the Arctic generally, and especially in the Chukchi Sea. The Canadian Arctic Shelf Exchange Study (CASES) project produced a high temporal resolution dataset of N pools and uptake in the southeast Beaufort Sea (Simpson et al. 2013; Tremblay et al. 2008), but there is no corresponding information for the Chukchi Sea. The only published reports of N uptake in the Chukchi Sea are offshore during late summer (Lee et al. 2012; Lee et al. 2007). Those studies found N uptake that was heavily reliant on regenerated forms of N, namely NH$_4^+$. This is in contrast to early studies of other regions of the Arctic (Daly et al. 1999; Garneau et al. 2007; Harrison et al. 1982; Kristiansen et al. 1994; Smith 1993) and implies an especially active microbial loop. So there is some indication that the Chukchi Sea is highly productive and fueled by N inputs, but little corresponding information on rates of N uptake and regeneration. This is especially true along the coast, where landfast sea ice and shallow waters limit the access to icebreakers.

The first goal of this work was to comprehensively understand N uptake and regeneration in the water column of the coastal Chukchi Sea. We focused on NH$_4^+$, NO$_3^-$, urea, and an amino acid mixture. In Chapter 2, I present data from $^{15}$N incubations done
to measure rates of N uptake and regeneration during January, April, and August of multiple phytoplankton size classes over two successive years. This allowed us to account for conditions of sea ice cover with no irradiance (winter), sea ice cover with high incident irradiance (spring), and high light and no sea ice cover (summer). Complementary data included ambient nutrient measurements of dissolved organic carbon and nitrogen, phosphate and silicate. Collaborators provided data on physical parameters such as temperature and salinity, along with measurements of bacterial production.

In uptake studies, size fractionation is commonly used to separate different phytoplankton size classes and also phytoplankton from bacteria. A drawback to this method is variable but significant bacterial retention on GF/F filters (Gasol and Morán 1999). An alternate method to distinguish phytoplankton from bacteria is to use flow cytometric sorting. In combination with $^{15}$N tracer incubations, uptake rates can to be unambiguously aligned with the microorganisms responsible for them (Lipschultz 1995; Lomas et al. 2011). After staining the samples, the bacterial fraction further separates into low nucleic acid (LNA) and high nucleic acid (HNA) groups (Gasol and del Giorgio 2000; Li et al. 1995). These bacterial groups are present whenever bacteria are investigated on a flow cytometer, regardless of region or protocol (Bouvier et al. 2007). Despite their ubiquity, their reason for existence and role in the environment is the subject of fierce debate (e.g Morán et al. 2007). Chapter 3 presents results of $^{15}$N uptake after sorting of LNA and HNA populations. This is the first time I am aware of that flow cytometric sorting of the LNA and HNA fractions have been used in combination with
\(^{15}\)N uptake. This work provides key insight into the activity of these bacterial subpopulations.

**Climate change**

Positive feedbacks within the Arctic are serving to accelerate the impacts of warming (Serreze and Francis 2006). Increases in air temperature are already leading to reduced volume and extent of sea ice, accelerated river discharge, rising sea level, thawing of permafrost, and coastal erosion (ACIA 2005; Anisimov et al. 2007). These physical changes in the Arctic are expected to have a profound effect on nutrients, temperature, and light attenuation, which are the fundamental drivers of microbial production. It is already known that changes in temperature and sea ice cover are impacting the timing and extent of community production in the coastal regions of the Arctic (Carmack and Wassmann 2006). What is not understood is how temperature increases will impact biogeochemical rate processes, although theory would predict that enzymatic processes would perform at higher rates in a warmer environment. Chapter 4 describes experiments undertaken to directly test the effect of temperature on rates of \(\text{NH}_4^+\) uptake and nitrification of coastal pelagic Arctic microorganisms.

**Sea ice**

Climate change is causing rapid changes in the sea ice landscape, with the Arctic predicted to become seasonally ice free by 2080 or earlier (e.g. Boé et al. 2009; Stroeve et al. 2007). Sea ice plays an important role in the region. Primary production on the
underside of sea ice may seed spring bloom populations in the spring (Perrette et al. 2011). Sea ice algae are productive both at the ice-water interface and also within the sea-ice matrix (Mock and Gradinger 1999). They provide a significant proportion of primary production in the Arctic Ocean and form the foundation of the food web in the both the pelagic and benthic realms (Brown and Belt 2012a, b; Riedel et al. 2007; Tremblay et al. 1989). Freshly produced organic matter decomposition from the receding ice during spring is also an important mechanism for the supply of surface nutrients (Conover et al. 1999; Cota et al. 1990).

While the effects of nutrient concentrations and light limitation on primary production in sea ice have been studied, the actual rates of N uptake and regeneration have received relatively less focus. In Chapter 5 I directly address the dearth of information on N uptake in Arctic sea ice. I was able to capture information on multiple depths within the ice, where I quantified uptake of $\text{NH}_4^+$ and $\text{NO}_3^-$ during January and April sampling trips. These processes are especially important in the highly productive coastal regions where first year landfast ice can support higher sympagic biomass (Gosselin et al. 1997).

**Literature cited**


Chapter 2 – Seasonal nitrogen uptake and regeneration in the western coastal Arctic
Abstract

The western coastal Arctic is subject to extreme seasonal changes in light, temperature, and nutrient inputs, all of which influence microbial resource competition, and therefore the structure of food webs in the region. This study investigated the relative importance of different nitrogen (N) compounds to a coastal Arctic microbial community. Using a dual $^{15}$N and $^{13}$C approach, we measured size-fractionated (> 3 μm and 0.7 - 3.0 μm) uptake rates of ammonium (NH$_4^+$), nitrate (NO$_3^-$), urea, and amino acids, along with primary productivity in the near shore environment of the Chukchi Sea. We performed incubations on seawater samples collected near Barrow, Alaska, during January, April, and August for two consecutive years. The total dissolved N (TDN) pool was dominated by NO$_3^-$ during the spring and winter (46-78% of TDN) but dropped to < 0.4 μmol N L$^{-1}$ (2-6% of TDN) during the late summer. Of the substrates tested, amino acids supported the bulk of N and C nutrition in both size fractions during ice-covered periods (winter and spring); urea uptake was low during these periods. During summer, absolute uptake of NH$_4^+$ was highest of the substrates tested, closely followed by urea and amino acids. The Chukchi Sea community is more reliant on NH$_4^+$ than NO$_3^-$, and NO$_3^-$ had the lowest relative preference index during any season. This is likely due to the availability of reduced N and the metabolic costs associated with reducing this oxidized form for use, although there is evidence of important late spring drawdown of NO$_3^-$ that coincides with the phytoplankton bloom period. Ours is the first study to investigate the importance of amino acid-derived N to Arctic near shore microbial communities and to compare N-cycling across winter, spring, and summer conditions. Reduced N forms
provide easily assimilated N to the microbial community and cannot be ignored in studies of Arctic N uptake dynamics.
Introduction

Historically, low temperatures were thought to inhibit microbial activity, however, several studies have shown that the Arctic has an abundant and well adapted community of psychrotolerant and psychrophilic (i.e. cold-loving) microorganisms (Connelly et al. 2006; Hodges et al. 2005; Yager et al. 2001), with high overall production near the coast (Bates et al. 2005). Even though microorganisms dominate biomass and production, and are critical to understanding the overall functioning of the ecosystem, microbial processes in the Arctic are currently understudied (Dyda et al. 2009; Kirchman et al. 2009a; Sherr et al. 2009). In the western coastal Arctic, primary and secondary production are principally supported by N inputs from the Pacific Ocean through the Bering Strait, with N drawn down to limiting levels during the summer when primary productivity is highest (Codispoti et al. 2005; Ortega-Retuerta et al. 2012). In contrast, heterotrophic bacterial communities are believed to be C limited throughout the year if algal production does not result in a surplus supply of dissolved organic matter (Kirchman et al. 2009a; Kirchman et al. 2005). Spring supplies of riverine dissolved organic matter may also provide labile material (Letscher et al. 2011) that is likely important to both primary and secondary producers (Kirchman et al. 2009b; Wickland et al. 2012). As such, the availability, uptake, and remineralization of N and C are subject to intense competition by autotrophic and heterotrophic microorganisms (Fouilland et al. 2007). This balance between autotrophs and heterotrophs is a fundamental driver of important ecological parameters in the marine environment and has important implications for food web dynamics and C exchange with the atmosphere.
As the amount of open water in the Arctic increases and freshens, primary productivity, especially along the coasts, is expected to increase because of increased light penetration (Arrigo et al. 2008; Pabi et al. 2008). If nutrient limitation is not alleviated by increased upwelling on the shelves (Carmack and Chapman 2003) or increased delivery of labile terrigenous material, primary production would likely be limited by N supply, thereby reducing the ability of the Arctic Ocean to act as a sink of carbon dioxide (Cai et al. 2010). With the rapid changes observed and predicted in the Arctic, a thorough understanding of N uptake and regeneration is needed to determine how the system is currently structured, and how it may react to changes in nutrient supply and removal processes.

Recent investigations of microbial population dynamics and productivity in the Arctic have shown that heterotrophs remain active during winter, even when they are decoupled from phytoplankton activity (Alonso-Sáez et al. 2008; Garneau et al. 2008). The logistical difficulties of sampling in this remote environment have limited studies of the nutrient concentrations and concomitant uptake supporting growth, especially over a full seasonal cycle, although there are some notable exceptions in the Canadian Arctic (Martin et al. 2012; Simpson et al. 2013a; Simpson et al. 2008; Tremblay et al. 2008). With months of no direct sunlight, temperatures that remain well below freezing for much of the year, and an unstable sea-ice landscape subject to shifting currents and winds, the majority of studies investigating N uptake have focused on areas amenable to research vessels and in the summer only. This project sought to quantify nutrient
inventories and size-fractionated uptake rates of N and C during winter, spring, and summer in the coastal waters of the Chukchi Sea near Barrow, Alaska.

**Methods**

*Field sample collection*

Sampling was performed during three seasons over two successive years centered on 71°21' N, 156°41' W, which is approximately 2.5 km northwest of Barrow, Alaska (Figure 1; Table 1). To capture the extreme Arctic light and physical conditions, sampling took place during winter (January 26-30, 2011 and January 16-21, 2012), spring (April 22-25, 2010 and April 26-May 2, 2011) and summer (August 25-29, 2010 and August 15-20, 2011). During winter and spring the sample site was covered by landfast ice and during the summer the site was in open water. Each of the sampling efforts included two trips to the sampling site and the data collected were averaged.

Winter and spring sampling was accomplished by traveling to near the outer edge of the landfast ice by snow machine and then cutting through the ice with an 8" auger to sample the seawater below. Once we accessed the water column, sample collection was performed from a tent placed above the hole. During the summer, when ice was not present at the site, sampling was accomplished from a small boat. Photosynthetically active radiation (PAR) was measured using a LI-193 Spherical Quantum Sensor (LI-COR Biosciences, Lincoln, NE) mounted on an extending arm to measure light levels under the ice (winter and spring) and to avoid shading from the boat during the summer. Depth profiles of temperature, salinity, and oxygen saturation were measured using a hand-deployed water quality sonde (Eureka Environmental, Austin, TX). Water samples
for analysis of depth profiles of chlorophyll a (Chl a), bacterial abundance and a suite of ambient nutrients were collected using a hand-deployed 1 L Niskin bottle. Larger volumes necessary for studies of N uptake were collected with a low-pressure submersible electric pump (Johnson Pump model #16004) powered either by a portable generator or a 12V battery. Sampling depths were chosen that were below any fresh water inputs from surface ice melt and above any contaminating inputs from the benthos (see Table 1).

*Laboratory incubations*

Water was collected into a series of 2 L acid-washed PETG bottles. All samples were run in duplicate and were inoculated with additions of $^{15}$N labeled ammonium chloride ($^{15}$NH$_4$Cl; 98.85% $^{15}$N), potassium nitrate (K$^{15}$NO$_3$; 98%), dual-labeled $^{15}$N- and $^{13}$C-urea (98%), or $^{15}$N and $^{13}$C labeled algal amino acid mixture comprised of 16 amino acids (96-99%; Cambridge Isotope Laboratories, Andover, MA). Labeled bicarbonate (H$^{13}$CO$_3^-$; Cambridge Isotope Laboratories) was also added to the same bottles of $^{15}$NH$_4$Cl and K$^{15}$NO$_3$ incubations. Literature values were used to estimate N tracer additions and alkalinity was used to derive ambient HCO$_3^-$ concentrations. During winter and spring, additions were made of 0.09, 0.4, 0.04, and 0.04 μmol N L$^{-1}$ for NH$_4^+$, NO$_3^-$, urea, and amino acids respectively. During summer, all incubations received 0.05 μmol N L$^{-1}$ of the relevant substrate. These additions corresponded to atom percent enrichments of 11% for both NH$_4^+$ and NO$_3^-$, 22% for urea, 29% for the amino acid mixture, and 7.3% for HCO$_3^-$ averaged across all seasons. The bottles were surrounded
by ambient seawater, placed in insulated coolers, and brought to the laboratory within one hour of collection to reduce temperature fluctuations. Separate bottles were filled in the same manner and used to track temperature at 60 second intervals with a HOBO TidbiT v2 water temperature data logger; fluctuations were limited to an overall average standard deviation of 0.31°C during all trips.

Samples were incubated for 24 hours at ambient light and temperature conditions in a temperature-controlled chamber. To mimic spectral attenuation and wavelength, light levels were maintained by fluorescent lights covered with GamColor blue films (GAM Products, Inc., Los Angeles, CA) and confirmed using the PAR sensor. At the termination of the incubations, the samples were filtered through 3.0 μm silver (Sterlitech Corporation) or Whatman GF/F (nominal pore size of 0.7 μm) filters. The filters were placed in cryovials and frozen until analysis. During this study, we measured bacterial abundance flow cytometrically before and after filtering through the GF/F filters, as they are known to capture bacteria as well as phytoplankton cells (Kirchman and Wheeler 1998). For analysis of total dissolved nitrogen (TDN) and dissolved organic carbon (DOC), a 40 mL aliquot of filtrate was poured into acid-washed and muffled glass EPA vials and immediately frozen; the remaining filtrate was poured into polypropylene tubes and frozen until analysis of the remaining nutrients.

Sample analyses

Concentrations of Chl a were estimated fluorometrically after acetone extraction using a Turner Design Model 10-AU fluorometer (Welschmeyer 1994). Bacterial
abundance was determined from triplicate whole water fixed in the field with additions of paraformaldehyde at a final concentration of 0.2% w/v, kept for 15 minutes at 5°C to ensure complete fixation, and stored at -80°C until laboratory analysis. Each sample was run in duplicate on a FACScalibur flow cytometer (Becton Dickinson, San Jose, CA) after staining with SYBR Green (Invitrogen) and the addition of reference beads (Spherotech, Fluorescent Yellow Particles, 1.7-2.2 μm), and then analyzed using FlowJo software (Treestar Inc., San Carlos, CA).

Concentrations of NH$_4^+$ were measured in triplicate using the phenol-hypochlorite method (Koroleff 1983). Duplicates of nitrate (NO$_3^-$), nitrite (NO$_2^-$), silicate (Si), and phosphate (PO$_4^{3-}$) were measured on a Lachat QuikChem 8500 autoanalyzer (Parsons et al. 1984). Dissolved primary amines (DPA), used as a proxy for amino acids (Keil and Kirchman 1991), were measured in triplicate on a Shimadzu RF-1501 spectrofluorometer following the o-phthaldialdehyde method (Parsons et al. 1984). Measurements of TDN and DOC were made in triplicate by high temperature combustion on a Shimadzu TOC-V TNM (Sharp et al. 2002). Dissolved organic nitrogen (DON) was calculated by subtracting inorganic N (NH$_4^+$, NO$_3^-$, and NO$_2^-$) from TDN (Bronk et al. 2000); the errors for all the terms were propagated to provide standard error for DON. Urea was measured in duplicate using the manual monoxime method (Price and Harrison 1987). Isotopic measurements for $^{15}$N and $^{13}$C uptake rate samples were run on a Europa GEO 20/20 mass spectrometer with an ANCA autosampler. N uptake rates were calculated as per Dugdale and Goering (1967), and C uptake rates as per Hama et al. (1983). The uptake rates associated with the 0.7–3.0 μm size fraction were estimated by subtraction of the
>3.0 μm fraction from rates measured from whole water collected on GF/F filters (nominal pore size = 0.7 μm). At the end of the incubations, we isolated NH$_4^+$ and NO$_3^-$ by solid phase extraction (Brzezinski 1987; Dudek et al. 1986) and the denitrifier method (Sigman et al. 2001) respectively. This allowed us to correct NH$_4^+$ uptake rates for isotope dilution (Glibert et al. 1982) and to calculate rates of NH$_4^+$ regeneration and nitrification. The isolation of NO$_3^-$ also included NO$_2^-$. As a result, the nitrification rates calculated are for NH$_4^+$ and NO$_2^-$ oxidation combined.

**Statistics**

Data analyses were conducted in the open-source statistical software program R, version 2.15.0 for Mac (R Core Team 2012). Mean rate estimates were compared using two-way analysis of variance (ANOVA) and Tukey's Honestly Significant Difference Method at the 95% confidence level. Linear regressions were plotted in SigmaPlot version 12.0 (Systat Software, San Jose, CA) and correlation coefficients (R values) ≥ 0.4 were considered significant. As described by McCarthy et al. (1977), the relative preference index (RPI) was calculated for each N substrate by size fraction to determine how the planktonic community responded to the different substrates during the different seasons.
Results

Physical conditions

Winter on the North Slope of Alaska is a time of near 24-hour darkness and the sampling site was fully ice covered, although the coastal landfast ice can be unstable until late winter. During January 2011, sampling took place the week after first sunrise (January 26 - 28). During this period there was less than four hours per day of direct sunlight with approximately two hours of twilight on either side of direct sun. In January 2012, sampling occurred the week prior to the first sunrise of the season (January 16-21). Both years, ice thickness was approximately 1.0 m (Table 1), and irradiance in the water column was < 0.30 μmol quanta\(^{-1}\) m\(^{-2}\) s\(^{-1}\). The water column was well mixed, as indicated by the constant temperature and salinity in the water column depth profiles (data not shown). Air temperatures ranged from -50 to -10°C, and the water temperature was -1.8°C. Salinity over the depth of the water column varied depending on the day of sampling and had a lower mean in 2011 (range = 30.7 - 31.6) than in 2012 (range = 33.6 - 34.2). Dissolved oxygen saturation averaged 87 ± 8 % during winter sampling.

During the spring sampling expeditions, the site experienced over 20 h d\(^{-1}\) of full sun. Air temperatures were higher (-18 to -7°C), but remained below freezing. Sea ice thickness averaged 1.0 m. As observed during the winter, the water column appeared to be well mixed with respect to temperature and salinity. Water temperature averaged -1.7 ± 0.07 °C and salinity ranged from 31.9 – 32.3. At the sampling depth used for all rate measurements, PAR was approximately 5 μmol quanta\(^{-1}\) m\(^{-2}\) s\(^{-1}\) with the exception of our
final April trip in 2011, when the PAR doubled to 11 μmol quanta⁻¹ m⁻² s⁻¹ because we sampled at a shallower depth (Table 1).

During the summer expeditions the sampling site experienced 17 – 19 hours d⁻¹ of sunlight, and air temperatures ranged from +8 to +19°C. As in other seasons the water column was well mixed with respect to temperature and salinity during August of 2010, with water column temperatures at +6.1°C. In 2011, there was a steady decrease in temperature from the surface (+5.6°C) to the bottom (+3.3°C). Salinity was 29.5 in 2010 and 31.5 in 2011. At the depth of sampling for the N uptake rate studies PAR was over 44 μmol quanta⁻¹ m⁻² s⁻¹, an order of magnitude higher than observed in the spring. Dissolved oxygen was supersaturated during summer, with a mean of 102 ± 1.7 %.

**Ambient biological abundance and nutrient concentrations**

Biological inventories were low during winter. Mean Chl a was 0.02 ± 0.01 μg L⁻¹ and bacterial cell counts averaged 2.9 ± 0.3 x10⁸ cells L⁻¹ across both sampling efforts. Concentrations of NH₄⁺ and NO₃⁻ were highest during winter (Table 2), with NO₃⁻ accounting for 46-78% of TDN. The winter of 2012 was the only season when NO₂⁻ was measurable, and even then was just barely above the detection limit (0.03 μmol N L⁻¹). Most of the DON (3.68 ± 0.89 μmol N L⁻¹) pool was not detected in the specific components that we measured; urea and DPA were only 8.5% of the total DON. With DOC concentrations of 79.2 ± 5.51 μmol C L⁻¹ during the winter, the DOC:DON ratio was 22.5 (Figure 2), well above the stochiometric ratio for plankton (Redfield 1934).
Both PO₄³⁻ and Si concentrations were approximately double relative to summer (Table 2).

Springtime algal biomass increased to an average of 0.55 ± 0.61 µg Chl a L⁻¹, but was variable from year to year, with 2010 values (range = 0.64 – 1.38 µg Chl a) 6-23 times higher than 2011 (range = 0.06 – 0.11 µg Chl a; Table 1). Bacterial cell counts remained about the same as winter at 2.1 ± 0.1 x10⁸ cells L⁻¹. Remaining high in spring, NO₃⁻ accounted for 50-68% of TDN (Table 2). Spring NH₄⁺ concentrations were approximately half of winter concentrations. Urea and DPA concentrations remained low in the spring while DON concentrations increased slightly to 4.78 ± 0.54 µmol N L⁻¹. Concentrations of DOC remained approximately the same as in winter on average at 79.0 ± 16.1 µmol C L⁻¹, but were variable over the two years of sampling. The nutrients PO₄³⁻ and Si remained high in spring, although Si varied by over a factor of two from 2010 to 2011.

During summer, Chl a increased to 0.63 ± 0.16 µg L⁻¹ and bacterial cell counts increased to 14.2 ± 13.0 x10⁸ cells L⁻¹. Both NO₃⁻ and NH₄⁺ concentrations were reduced compared to the prior season. Exhibiting the opposite pattern, DON was relatively higher during summer (6.59 ± 0.75 µmol N L⁻¹). Urea and DPA concentrations increased in absolute and relative (9.8% of total DON) terms, but remained below 0.5 µmol N L⁻¹. Concentrations of DOC also increased, to 92.3 ± 5.87 µmol C L⁻¹, but not as much relative to DON, such that the DOC:DON ratio fell to 14.1, which is still well above the canonical Redfield ratio, but almost half of the ratio found during winter. Concentrations of PO₄ dropped during summer to ~47% of concentrations present during the ice-covered
seasons. Similarly, Si was even lower relative to other seasons, drawn down to only 25% of winter and spring levels.

Uptake rates

Our check on the size fractionation technique revealed that some bacteria were retained on the GF/F filters, but the percentages of bacterial cells retained differed depending on the season. During summer, 61% of the bacterial cells were retained, and even more (74%) during spring, while winter apparently shifted to smaller cells or less aggregates and the GF/F filters only retained 36% of the bacterial cells. Thus, our N and C uptake rates include variable but incomplete contributions from heterotrophic bacteria. During September in the surface waters of a Baffin Bay polynya, Fouilland et al. (2007) calculated that GF/F filters captured 80% of the total living bacterial community, which roughly aligns with our results for August.

Low biomass in the winter samples resulted in the lowest absolute N uptake rates (Figure 3). Within the winter sampling season, there was no significant difference (p > 0.05) between the >3 μm and 0.7-3.0 μm uptake rates of NH₄⁺ (Table 3). Even though NO₃⁻ concentrations were high, total uptake was lower than NH₄⁺, and the 0.7-3.0 μm fraction had higher rates than the larger (>3.0 μm) fraction. Urea uptake rates were lower than the other substrates, even though ambient urea concentrations remained essentially unchanged throughout the year. Rates of amino acid derived N (AAₙ) uptake were highest of all the substrates measured in both size fractions; again the uptake rates in the smaller size fraction was higher than those in the larger size fraction. The amino acid
mixture also appears to provide energy in the form of C for the smaller size fraction (Figure 4). During the winter season, uptake of C from the amino acid mixture (AAc) was highly variable between the two years (Table 3). Uptake of C from urea (UreaC) and HCO₃⁻ uptake was not detected in any of the winter samples.

All of the spring rates were approximately double the winter rates, and exhibited a similar relative pattern to winter. For all of the substrates, there were no statistically significant differences (p > 0.05) in the rates between the size fractions (Figure 3). During the spring, NH₄⁺ continued to be an important resource, for both the small and large size fractions. As in the winter, even though NO₃⁻ and urea concentrations were high, uptake rates were still < 0.01 nmol N L⁻¹ h⁻¹. Amino acids remained the preferred form of N for uptake, especially for the smaller size fraction that exhibited AAₙ uptake rates approximately three times higher than the larger size fraction. Uptake of UreaC was not detected (Figure 4) and uptake rates of HCO₃⁻ were not significantly different (p > 0.05) between the size fractions. Absolute uptake of AAc and HCO₃⁻ were approximately equal in the larger size fraction (> 3.0 μm). The highest C uptake was from the smaller size fraction AAc, although it was highly variable both within a season and from year to year (Table 3).

During summer, absolute uptake rates for all substrates except AAN were more than an order of magnitude higher than the other two seasons, with NH₄⁺ utilization being the highest relative to other substrates (Figure 3). Unlike the ice covered winter and spring seasons, the larger size fraction had higher uptake rates. The uptake of NH₄⁺ dominated both the smaller and larger size fractions. The >3.0 μm size fraction jumped
two orders of magnitude for NO$_3^-$ uptake and the 0.7-3.0 µm fraction also increased by the same magnitude, but only during 2011. During 2012, there was no difference between the spring and summer NO$_3^-$ uptake rates for the smaller size fraction. Rates of urea uptake also increased by more than an order of magnitude, and had the second highest rates beside NH$_4^+$ uptake. Compared to spring AAc uptake doubled in both size fractions and remained an important N source, but did not have the same relative seasonal increase as the other substrates. Uptake of AAc remained the highest of the C sources provided. Uptake of HCO$_3^-$ increased by an order of magnitude for both size fractions. Urea$_C$ utilization was only detected during the summer 2010 sampling (Table 3), making the seasonal average highly variable.

It was possible to use the calculated RPI to define the hierarchical preference of various substrates relative to their availability (McCarthy et al. 1977). For both winter and spring, the RPI of AAn was more than an order of magnitude higher than the other N substrates (Table 4). While this method is known to be sensitive to nutrient concentration (Stolte and Riegman 1996), DPA concentrations remain low year-round, and cannot explain the seasonal difference. The RPI also shows that urea was typically preferred over NO$_3^-$ when it was available.

**Nutrient Regeneration**

Rates of NH$_4^+$ regeneration averaged 13.4 ± 7.4 nmol N L$^{-1}$ h$^{-1}$ for all samples, but with large interannual variability during the spring (Table 5). Nitrification was high during the winter and spring (mean of 21.7 ± 10.9 nmol N L$^{-1}$ h$^{-1}$), but dropped more than
an order of magnitude during summer. During this study, \( \text{NO}_3^- \) concentrations were high during winter and spring, and no regeneration of \( \text{NO}_3^- \) was detected.

**Discussion**

Most Arctic marine N cycling studies have focused on dissolved inorganic N (\( \text{NH}_4^+, \text{NO}_3^- \)) with limited investigations of the role of DON sources such as urea and amino acids. Additionally, studies have generally been conducted in offshore waters and during the summer (Table 6). This is the first study we are aware of that provides comprehensive N uptake rates in Arctic near shore waters during winter, spring, and summer, each of which present a unique set of environmental conditions. In addition to variations in seasonal light and ice coverage, episodic advection can change the nutrient regime rapidly on the coastal shelves (Carmack and Chapman 2003). This leads to high regional (Codispoti et al. 1991) and interannual (Kirchman et al. 2009a) variability in community production, along with dynamic winter to summer changes in community composition (Alonso-Sáez et al. 2008). Corresponding to changes in the physical and biological realms, it would be expected that nutrient inventories are also highly dynamic. Biogeochemical cycles both drive biological variables and respond to changes in the system over the yearly cycle. The seasonal sampling allowed us to quantify N under conditions at the extremes of light and temperature present in the region, as the confounding factors of temperature, irradiance, and nutrient concentrations have historically eluded straightforward description (Smith and Harrison 1991).
Seasonal trends

By January, TDN accumulated to seasonal highs. Although NO$_3^-$ was the most abundant N pool, NO$_3^-$ uptake rates were consistently lower than that of NH$_4^+$ and AA$_N$. This is likely due to the metabolic cost needed to reduce NO$_3^-$ to NH$_4^+$ intracellularly. Amino acids accounted for the majority of uptake for both N and C of the substrates tested, indicating that microbial activity was primarily supported by DON sources during the winter. Interestingly, there was evidence of the larger size fraction also taking up N, but there is no concomitant evidence of HCO$_3^-$ uptake. Given that there was little to no light available, it is not surprising that the smaller size fraction is active, albeit at reduced rates. What is surprising is that the larger size fraction, which presumably consists of large phytoplankton, took up amino acids at all. There have been reports of mixotrophy in this system (Cottrell and Kirchman 2009; Seuthe et al. 2011), and there is mounting evidence that phytoplankton can effectively utilize DON (e.g. Berman and Bronk 2003; Bronk et al. 2007). Alternatively, it could be that particle-attached bacteria and other microheterotrophs (e.g. microflagellates, small ciliates) were collected on the 3.0 μm filters.

Although surface irradiance increased dramatically in April, ice thickness continued to grow toward its yearly apex. The water column community remained light limited, and NO$_3^-$ uptake was low despite near seasonally high NO$_3^-$ concentrations. In fact, NO$_3^-$ utilization was low year round, but especially so during winter and spring. This observation is consistent with earlier work that found NO$_3^-$ uptake to be more sensitive to low temperatures than NH$_4^+$ (Reay et al. 1999). Our winter and spring NO$_3^-$
uptake rates are similar to the only other study of N uptake in under-ice plankton of this region, where NO$_3^-$ uptake rates ranged from 0.02 – 0.39 nmol N L$^{-1}$ h$^{-1}$ (Lee et al. 2010; Table 6). Though still cold in spring with relatively low PAR, winter to spring Chl $a$ increased by a factor of 23, and rates of NH$_4^+$ and AAN uptake increased by over a factor of two overall. These increases in rates occurred despite little difference in the ambient nutrient or temperature regimes from winter to spring, implying light as the critical limiting factor during winter and spring in this system (Terrado et al. 2008; Tremblay et al. 2006).

As Garneau et al. (2007) showed, the changes in uptake rates between ice-free and ice-covered conditions can happen quickly and the difference can be large (Table 6). When light limitation imposed by the sea ice was completely lifted in the summer, the biological community responded with increased biomass and broad usage of all the N substrates measured (NH$_4^+$, NO$_3^-$, urea, and AAN). Ambient DIN was drawn down below 1.0 µmol N L$^{-1}$, while DON rose in concentration to over 6.0 µmol N L$^{-1}$ (Figure 2). This rapid drawdown of DIN and production of DON, coupled with increases in Chl $a$ and NO$_3^-$ uptake rates (Table 3) all point to high levels of productivity over the summer. Uptake rates of all N substrates except AAN increased by an order of magnitude during the summer. As shown by the RPI results (Table 4), rather than AAN being the dominant preferred substrate, all of the substrates are utilized to a similar degree during August. The phytoplankton community was finally able to utilize the available light for photosynthesis and incorporate N from NO$_3^-$, as the cost of reduction may be less than costs of competition for scarce amino acid compounds. In a study spanning spring to late
fall in the Canadian Arctic, Martin et al. (2012) found higher dependence on NO\textsubscript{3}\textsuperscript{-} (relative to NH\textsubscript{4}\textsuperscript{+}) only during early spring. With time-series sampling in the Amundsen Gulf (Canadian Arctic), Simpson et al. (2013a) found that indeed absolute uptake rates of NO\textsubscript{3}\textsuperscript{-} were only greater than NH\textsubscript{4}\textsuperscript{+} and urea uptake during a bloom (Table 6). Our sampling bracketed the water column spring bloom period. Given the low concentrations of NO\textsubscript{3}\textsuperscript{-} (<0.4 \mu mol N L\textsuperscript{-1}) that we found in August, it is clear that the period of most intense NO\textsubscript{3}\textsuperscript{-} drawdown and high Chl \textit{a} was over (e.g. Yager et al. 2001) by the time our sampling began. We did not correct our NO\textsubscript{3}\textsuperscript{-} uptake rates for isotope dilution, as effects are generally negligible when ambient concentrations are high (as it was during January and April), although the effect of isotope dilution on NO\textsubscript{3}\textsuperscript{-} uptake is not well understood because there are far fewer measurements available (Harrison et al. 1985).

During late August of both study years, Chl \textit{a} concentrations were relatively low (<1.0 \mu g/L; Table 2) compared to temperate regions, but there was a 52 – 81% reduction in the concentration of Si from April to August (Figure 2), indicating the presence of diatoms. In addition, as light limitation was lifted by the seasonal cycle and retreat of sea ice, C uptake from AA\textsubscript{C} increased by a factor of seven while HCO\textsubscript{3}\textsuperscript{-} uptake increased by a factor of 60 from winter/spring to summer. It should be noted that both size fractions took up HCO\textsubscript{3}\textsuperscript{-} in summer. This could indicate that there are small chemoautotrophs or photoautotrophs (Worden and Not 2008) passing through the 3.0 \mu m filters. Another potential source of C could be Urea\textsubscript{C}, but uptake rates from this study are relatively low, which is consistent with the results of Harrison et al. (1985) who found that Urea\textsubscript{C} assimilation was highest at more northerly sites (> 77° N) in their study of Baffin Bay.
The coastal area of the Chukchi Sea is highly productive (Bates et al. 2005), with high relative uptake rates of N to support primary and secondary production. During our August sampling, we measured total absolute N uptake (i.e. uptake of NH$_4^+$, NO$_3^-$, and urea combined) higher than those found in almost any other N uptake study in the western Arctic (Table 6). Additionally, the general trend of studies from other regions of the western Arctic is that NO$_3^-$ uptake rates are generally equal to or greater than NH$_4^+$ uptake, with more importance ascribed to "new" as opposed to "regenerated" production (cf. Dugdale and Goering 1967). This study and others in the region (Lee et al. 2012; Lee et al. 2007) are the only consistent results from the western Arctic that show a preference for NH$_4^+$ over NO$_3^-$ (Table 6). This trend continues during the January and April sampling periods. It is therefore unlikely that it is simply a matter of substrate availability, but rather an indication of a fundamental difference in the metabolic strategies of the plankton community in the coastal Chukchi Sea.

The importance of DON

Many of the earliest studies of N uptake in the Arctic took place during the summer in the large polynyas surrounding Greenland and, as stated above, found that NH$_4^+$ and NO$_3^-$ uptake were of similar magnitude (Table 6). When measured, urea uptake was generally a small component (~15% of the total uptake) in those regions. This study is the only one we are aware of in this region that measured urea uptake, and found that it accounted for 27% of the uptake (not including AA$_N$, for a direct comparison to the other studies). The uptake rates reported here are on the upper end of the range reported
by others despite the use of tracer additions. However, Martin et al. (2012) in their studies explicitly investigated the significance of saturating additions with inorganic N in Arctic waters and reported them to be minimal. The relatively high N uptake rates observed in this study may be explained by being conducted in coastal rather than open ocean waters. Coastal systems are known to be highly productive, especially when compared to pelagic systems that are further removed from allochthonous nutrient sources (e.g. rivers, Bering Strait).

In the other studies of the western Arctic, RPIs have indicated low preference for urea even when uptake rates are high (Harrison et al. 1985), although urea concentrations are highly variable throughout the region (Simpson et al. 2008). At this study site in the western Arctic, the community present also appears to favor NH$_4^+$ and AA$_N$ utilization (Table 4). Only during the summer, when zooplankton and migrating seabirds are a likely localized source of urea via excretion and sloppy feeding (Conover and Gustavson 1999), does the microbial community seem to respond. However, the source is not as important as overall N concentrations, competition for them, and the need to utilize a range of substrates. In fact, urea concentrations are also fairly high during the spring, most likely due to release from landfast ice (Conover et al. 1999), but uptake rates remain low.

During both the winter and spring AA$_N$ was the most preferred form of N (Table 4). This is consistent with previous reports from the Arctic that demonstrated that labile dissolved organic matter constituents including amino acids are an important nutrient resource for prokaryotes (Alonso-Sáez et al. 2008; Kirchman et al. 2007; Nikrad et al.
2012; Rich et al. 1997; Yager and Deming 1999). In marine systems generally, amino acids can support up to 40% of bacterial production (Kirchman 2000) and prokaryotes can assimilate them even at nanomolar concentrations (Cottrell and Kirchman 2000; Ouverney and Fuhrman 2000). Pelagic microorganisms in the Northeast Water polynya (Greenland) had high specific affinities for amino acid mixtures even at subzero temperatures (Yager and Deming 1999). In this study, AAN uptake was observed year-round, with decreased importance relative to other N and C sources in the summer. During the winter of 2012, the molar C:N uptake ratio of the amino acid mixture was 10.5 ± 5.4 for the whole community, but for all other sampling trips was 2.6 ± 1.0. The mixture added had an initial C:N of 3.7, highlighting the use of the amino acid mixture as a rich N source that the microbial community is able to use efficiently. The fact that AAN was being taken up by both larger (>3.0 μm) and smaller (0.7 – 3.0 μm) cells lends further credence to the importance of DON as a nutrient source for phytoplankton as well as bacteria, along with potential uptake by microflagellates and ciliates (Bronk et al. 2007; Sanderson et al. 2008).

In the future, the components and lability of Arctic DON is expected to change as more terrigenous material is transported from the tundra to the coastal ocean (Sipler et al. in prep.; Tait et al. submitted). It is therefore important to understand how the concentration of DON components like urea and amino acids are changing over time, as that will have a large impact on the processing of this material. In the Arctic, rivers currently deliver high concentrations of labile organic nutrients (e.g. Dittmar and Kattner 2003; Holmes et al. 2008; McClelland et al. 2012). DON tends to be rapidly consumed
in the marine receiving waters, but can also be locally produced in the Chukchi Sea (Letscher et al. 2013). In this study, urea and AA\textsubscript{N} were only a small percentage (<10%) of the overall DON pool, but proved to have high rates of uptake compared to inorganic N. Marine DON consists of a complex mixture of compounds that remain uncharacterized, but urea and amino acids are highly labile fractions (Bronk 2002). Our results highlight the importance of the quality of the DON pool, not just the quantity, when trying to assess the complex overall dynamics of DON production and consumption in the Arctic.

*The importance of regeneration*

Regeneration of N sources can imply tight coupling within a system. In the summer, when NH\textsubscript{4}\textsuperscript{+} resources are scarce, we measured relatively high regeneration rates (Table 5), but the relationship between NH\textsubscript{4}\textsuperscript{+} concentrations and regeneration rates is highly equivocal, with no significant correlation (Figure 5; \(R^2 = 0.21\)) between them. While the overall trend appears to be positive, regeneration rates during the summer seem to generally confound this trend, such that even though ambient concentrations are low, regeneration rates are relatively high, indicating a tightly coupled system during summer that helps to keep NH\textsubscript{4}\textsuperscript{+} concentrations low (Clark et al. 2008).

In addition to regeneration, nitrification could oxidize a significant portion of NH\textsubscript{4}\textsuperscript{+} to NO\textsubscript{3}\textsuperscript{−}, especially during the ice-covered seasons (Christman et al. 2011, Veuger et al. 2013). In the Southern Ocean, Bianchi et al. (1997) measured spring nitrification rates of 1.7 – 3.5 nmol L\textsuperscript{−1} h\textsuperscript{−1}. Our late summer rates (mean = 1.02 ± 0.32 nmol L\textsuperscript{−1} h\textsuperscript{−1};
Table 5) were below that range, but during winter and spring we measured nitrification rates more than 20 times higher than summer, with a mean of $21.7 \pm 10.9 \text{ nmol L}^{-1} \text{ h}^{-1}$. This seasonal disparity aligns with recent work in the same region, where Christman et al. (2011) measured mean nitrification rates of 3.6 and 89.2 nmol L$^{-1}$ h$^{-1}$ during summer and winter respectively. During winter and early spring, Simpson et al. (2013b) calculated a mean nitrification rate of $317 \pm 7.2 \text{ nmol L}^{-1} \text{ h}^{-1}$ in the Beaufort Sea. In the seasonally ice covered North Sea (Netherlands), Veuger et al. (2013) measured mean nitrification rates of $93.8 \pm 54.1$ (range = 43 – 221) nmol L$^{-1}$ h$^{-1}$ during that same time period. It is clear that nitrification is very active during winter relative to summer and could explain the high NO$_3^-$ inventories found generally in the Arctic before the spring bloom period (Tremblay et al. 2008). In addition to the seasonal dynamics in light and temperature that can influence nitrification rates, there is some evidence that the process is sensitive to NH$_4^+$ concentrations (Ward 2008). In this study nitrification rates were significantly correlated with ambient NH$_4^+$ concentrations (Figure 5; $R^2 = 0.54; p = 0.0005$), in contrast to urea concentrations ($R^2 = 0.18; p > 0.05$) which have been proposed as an important source for nitrification in the polar regions (Alonso-Sáez et al. 2012).

Nitrification dominates during the low-light seasons, and the NH$_4^+$ assimilation during summer (Veuger et al. 2013), which is likely a result of light inhibition of nitrification (Ward 2008). Additionally, the low concentration of NH$_4^+$ during summer could result in phytoplankton and bacteria effectively outcompeting nitrifiers for substrate.
Conclusions

The western coastal Arctic is shifting to a regime that is increasingly reliant on pelagic processes (Grebmeier et al. 2006). The contribution of the sea ice community to the ecology of the region is sure to decline along with the sea ice habitat. At least in the short term, pelagic phytoplankton will respond positively to the earlier light penetration through the ice (Arrigo et al. 2012; Lee et al. 2011) and open water conditions (Arrigo et al. 2008). In the future, the Arctic Ocean is generally expected to be heavily influenced by increased thermal stratification (Peterson et al. 2006). However, the near shore site occupied by this study was never thermally stratified, likely due to strong currents, and therefore the physical forcing mechanisms in the shallow coastal areas may respond to other physical processes or not at all. Our work here highlights instead the unique characteristics of the near shore environment of the Chukchi Sea. While there is likely a short-term pulse of \( \text{NO}_3^- \) uptake that corresponds with the spring bloom period (e.g. Martin et al. 2012), the remainder of the year is heavily dependent on \( \text{NH}_4^+ \) and dissolved organic matter sources. This highly regenerative system will likely limit benthic coupling and the ability of the coastal region to act as a C sink. By focusing on offshore locations during summer, other studies have not been able to elucidate the unique conditions and activity of the near shore environment and therefore may misrepresent the biogeochemical dynamics of the Chukchi Sea and the Arctic Ocean as a whole. It will be critical to incorporate seasonal uptake rates of N and certain C containing compounds in the western Arctic shallow coastal zone, which display different importance of N compounds than previously studied areas in the Arctic.
Our research indicates that there is a robust planktonic microbial community during winter that has access to large pools of ambient N, but maintains low levels of assimilation until the lifting of light limitation in the summer. The microbial community is able to respond to nutrient availability and utilize multiple forms of both N and C for low levels of growth and maintenance during the ice-covered seasons. We have demonstrated that specific DON components play a large role in the N cycling processes of the coastal western Chukchi Sea. It will be important to understand how future changes in DON delivery and the reduction of sea ice will interplay to drive the productivity of this system.

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Tables

Table 1. Physical conditions of the individual sampling locations. Exact locations and physical parameters for stations in this study. During ice-covered seasons, sample depth was measured from the bottom of the sea ice. Nd is for no data.

<table>
<thead>
<tr>
<th>Month</th>
<th>Day</th>
<th>Year</th>
<th>Latitude (W)</th>
<th>Longitude (N)</th>
<th>Water depth (m)</th>
<th>Sample depth (m)</th>
<th>Ice depth (m)</th>
<th>PAR (µmol quanta⁻¹ m⁻² s⁻¹)</th>
<th>Temp. (°C)</th>
<th>Salinity</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>26</td>
<td>2011</td>
<td>156°41'40&quot;</td>
<td>71°20'21&quot;</td>
<td>10.4</td>
<td>2.0</td>
<td>0.91</td>
<td>0.30</td>
<td>-2.5</td>
<td>26.2</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>2011</td>
<td>156°41'40&quot;</td>
<td>71°20'21&quot;</td>
<td>10.4</td>
<td>2.0</td>
<td>0.91</td>
<td>0.30</td>
<td>-2.0</td>
<td>32.9</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>2012</td>
<td>156°41'00&quot;</td>
<td>71°21'20&quot;</td>
<td>16.5</td>
<td>2.0</td>
<td>1.20</td>
<td>0.08</td>
<td>-1.8</td>
<td>33.5</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td></td>
<td>156°34'33&quot;</td>
<td>71°22'12&quot;</td>
<td>7.0</td>
<td>1.0</td>
<td>0.56</td>
<td>0.12</td>
<td>-1.9</td>
<td>33.8</td>
</tr>
<tr>
<td>April</td>
<td>23</td>
<td>2010</td>
<td>156°41'09&quot;</td>
<td>71°21'22&quot;</td>
<td>17.7</td>
<td>8.0</td>
<td>0.55</td>
<td>5.0</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td></td>
<td>156°41'09&quot;</td>
<td>71°21'22&quot;</td>
<td>17.7</td>
<td>8.0</td>
<td>0.55</td>
<td>5.0</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>2011</td>
<td>156°43'16&quot;</td>
<td>71°18'07&quot;</td>
<td>17.0</td>
<td>6.5</td>
<td>1.50</td>
<td>4.7</td>
<td>-1.6</td>
<td>31.5</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td></td>
<td>156°43'39&quot;</td>
<td>71°18'13&quot;</td>
<td>8.0</td>
<td>4.0</td>
<td>1.30</td>
<td>11</td>
<td>-1.8</td>
<td>32.4</td>
</tr>
<tr>
<td>August</td>
<td>25</td>
<td>2010</td>
<td>156°41'09&quot;</td>
<td>71°21'22&quot;</td>
<td>12.5</td>
<td>4.0</td>
<td>0.0</td>
<td>65</td>
<td>6.0</td>
<td>31.4</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td></td>
<td>156°41'09&quot;</td>
<td>71°21'22&quot;</td>
<td>12.5</td>
<td>4.0</td>
<td>0.0</td>
<td>44</td>
<td>6.0</td>
<td>31.6</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>2011</td>
<td>156°43'16&quot;</td>
<td>71°18'07&quot;</td>
<td>17.0</td>
<td>4.0</td>
<td>0.0</td>
<td>nd</td>
<td>5.9</td>
<td>30.2</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td></td>
<td>156°43'16&quot;</td>
<td>71°18'07&quot;</td>
<td>17.0</td>
<td>8.0</td>
<td>0.0</td>
<td>nd</td>
<td>4.7</td>
<td>30.6</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td></td>
<td>156°43'39&quot;</td>
<td>71°18'13&quot;</td>
<td>8.0</td>
<td>2.0</td>
<td>0.0</td>
<td>nd</td>
<td>4.7</td>
<td>30.2</td>
</tr>
</tbody>
</table>
Table 2. Ambient biological and chemical inventories. For each sampling trip, which consisted of at least two visits to the sampling site, the overall mean and standard deviation of Chl $\alpha$, bacterial abundance, and nutrient concentrations are given for the depth at which uptake incubations were performed (see Table 1).

<table>
<thead>
<tr>
<th>Month</th>
<th>Year</th>
<th>Chl $\alpha$ $\mu g$ L$^{-1}$</th>
<th>Bacterial Abundance $10^8$ cells L$^{-1}$</th>
<th>NH$_4^+$ $\mu mol$ N L$^{-1}$</th>
<th>NO$_3^-$ $\mu mol$ N L$^{-1}$</th>
<th>Urea $\mu mol$ N L$^{-1}$</th>
<th>$'DPA'$ $\mu mol$ P L$^{-1}$</th>
<th>PO$_4^{3-}$ $\mu mol$ Si L$^{-1}$</th>
<th>Si $\mu mol$ C L$^{-1}$</th>
<th>DOC $\mu mol$ P L$^{-1}$</th>
<th>DON $\mu mol$ N L$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>January 2011</td>
<td>0.03 ± 0.00</td>
<td>2.45 ± 0.60</td>
<td>6.93 ± 1.03</td>
<td>0.25 ± 0.05</td>
<td>0.14 ± 0.04</td>
<td>1.35 ± 0.08</td>
<td>20.0 ± 2.9</td>
<td>75.3 ± 2.24</td>
<td>4.3 ± 0.18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>January 2012</td>
<td>0.02 ± 0.01</td>
<td>2.8 ± 0.3</td>
<td>0.69 ± 0.24</td>
<td>9.07 ± 3.11</td>
<td>0.17 ± 0.04</td>
<td>0.07 ± 0.01</td>
<td>1.28 ± 0.19</td>
<td>28.4 ± 8.3</td>
<td>83.1 ± 1.81</td>
<td>3.1 ± 0.42</td>
<td></td>
</tr>
<tr>
<td>April 2010</td>
<td>1.01 ± 0.52</td>
<td>0.69 ± 0.11</td>
<td>5.91 ± 1.25</td>
<td>0.38 ± 0.39</td>
<td>0.15 ± 0.14</td>
<td>0.98 ± 0.04</td>
<td>10.8 ± 1.3</td>
<td>90.4 ± 7.31</td>
<td>5.2 ± 1.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>April 2011</td>
<td>0.09 ± 0.03</td>
<td>3.2 ± 0.9</td>
<td>0.86 ± 0.37</td>
<td>9.46 ± 1.69</td>
<td>0.20 ± 0.01</td>
<td>0.10 ± 0.02</td>
<td>1.15 ± 0.17</td>
<td>34.0 ± 7.3</td>
<td>67.6 ± 0.33</td>
<td>4.4 ± 0.24</td>
<td></td>
</tr>
<tr>
<td>August 2010</td>
<td>1.38 ± 1.17</td>
<td>5.9 ± 3.8</td>
<td>0.37 ± 0.11</td>
<td>0.16 ± 0.04</td>
<td>0.35 ± 0.16</td>
<td>0.20 ± 0.07</td>
<td>0.59 ± 0.04</td>
<td>5.14 ± 1.1</td>
<td>96.5 ± 3.95</td>
<td>6.1 ± 0.52</td>
<td></td>
</tr>
<tr>
<td>August 2011</td>
<td>0.52 ± 0.18</td>
<td>19.7 ± 14.7</td>
<td>0.80 ± 0.50</td>
<td>0.35 ± 0.07</td>
<td>0.49 ± 0.33</td>
<td>0.26 ± 0.15</td>
<td>0.52 ± 0.04</td>
<td>6.46 ± 1.9</td>
<td>88.2 ± 6.55</td>
<td>7.11 ± 1.5</td>
<td></td>
</tr>
</tbody>
</table>

$'DPA'$ Dissolved primary amines
Table 3. Nitrogen and carbon absolute uptake rates. Mean and standard deviations of N and C uptake rates in the large (>3.0 μm) and small (0.7 to 3.0 μm) size fractions. BDL is below detection limit.

<table>
<thead>
<tr>
<th>Month</th>
<th>Year</th>
<th>Fraction</th>
<th>$\text{NH}_4^+$ (nmol N L$^{-1}$ h$^{-1}$)</th>
<th>$\text{NO}_3^-$ (nmol N L$^{-1}$ h$^{-1}$)</th>
<th>Urea (nmol N L$^{-1}$ h$^{-1}$)</th>
<th>$^{1}\text{AA}_N$ (nmol N L$^{-1}$ h$^{-1}$)</th>
<th>$^{1}\text{AA}_C$ (nmol C L$^{-1}$ h$^{-1}$)</th>
<th>$\text{HCO}_3^-$ (nmol C L$^{-1}$ h$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>2011</td>
<td>&gt; 3 μm</td>
<td>0.16 ± 0.01</td>
<td>0.05 ± 0.01</td>
<td>0.01 ± 0.00</td>
<td>0.21 ± 0.14</td>
<td>0.57 ± 0.46</td>
<td>BDL</td>
</tr>
<tr>
<td></td>
<td>2012</td>
<td>&gt; 3 μm</td>
<td>0.07 ± 0.00</td>
<td>0.06 ± 0.02</td>
<td>0.01 ± 0.01</td>
<td>0.11 ± 0.09</td>
<td>0.08 ± 0.11</td>
<td>BDL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.7-3 μm</td>
<td>0.06 ± 0.06</td>
<td>0.14 ± 0.02</td>
<td>0.01 ± 0.01</td>
<td>0.34 ± 0.26</td>
<td>3.74 ± 5.50</td>
<td>BDL</td>
</tr>
<tr>
<td>April</td>
<td>2010</td>
<td>&gt; 5 μm</td>
<td>0.36 ± 0.00</td>
<td>0.09 ± 0.07</td>
<td>0.18 ± 0.18</td>
<td>0.11 ± 0.13</td>
<td>0.33 ± 0.42</td>
<td>0.71 ± 0.62</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.2-5 μm</td>
<td>0.20 ± 0.67</td>
<td>0.01 ± 0.02</td>
<td>0.06 ± 0.07</td>
<td>0.09 ± 0.02</td>
<td>0.47 ± 0.67</td>
<td>0.27 ± 0.45</td>
</tr>
<tr>
<td></td>
<td>2011</td>
<td>&gt; 3 μm</td>
<td>0.28 ± 0.16</td>
<td>0.09 ± 0.04</td>
<td>0.02 ± 0.00</td>
<td>0.45 ± 0.37</td>
<td>0.62 ± 0.53</td>
<td>0.13 ± 0.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.7-3 μm</td>
<td>0.61 ± 0.18</td>
<td>0.19 ± 0.07</td>
<td>0.02 ± 0.00</td>
<td>1.27 ± 0.69</td>
<td>2.01 ± 1.09</td>
<td>0.14 ± 0.03</td>
</tr>
<tr>
<td>August</td>
<td>2010</td>
<td>&gt; 3 μm</td>
<td>10.7 ± 3.70</td>
<td>2.79 ± 0.06</td>
<td>4.92 ± 2.79</td>
<td>1.42 ± 0.15</td>
<td>2.26 ± 0.83</td>
<td>20.4 ± 0.36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.7-3 μm</td>
<td>8.61 ± 6.56</td>
<td>1.25 ± 0.80</td>
<td>3.71 ± 0.83</td>
<td>3.80 ± 1.65</td>
<td>7.25 ± 10.3</td>
<td>4.42 ± 6.25</td>
</tr>
<tr>
<td></td>
<td>2011</td>
<td>&gt; 3 μm</td>
<td>15.5 ± 8.07</td>
<td>1.75 ± 1.48</td>
<td>4.69 ± 4.89</td>
<td>2.93 ± 1.58</td>
<td>9.95 ± 6.59</td>
<td>42.3 ± 25.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.7-3 μm</td>
<td>3.52 ± 4.22</td>
<td>0.17 ± 0.15</td>
<td>1.34 ± 1.89</td>
<td>2.51 ± 1.31</td>
<td>4.26 ± 6.77</td>
<td>4.47 ± 2.92</td>
</tr>
</tbody>
</table>

$^{1}\text{AA}_N$ and $\text{AA}_C$ refers to rates uptake of the nitrogen and carbon component of amino acids, respectively.
Table 4. **Relative preference index of N substrates.** For each substrate, the mean and standard deviation for RPI for each season and year. Rates used in the RPI calculation were for the whole community collected on GF/F filters (i.e. the 3.0 μm fraction was not subtracted from the overall rate). Means are presented for each season.

<table>
<thead>
<tr>
<th>Month</th>
<th>Year</th>
<th>NH₄⁺</th>
<th>NO₃⁻</th>
<th>Urea</th>
<th>AA_N</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>2011</td>
<td>1.3 ± 0.3</td>
<td>0.2 ± 0.0</td>
<td>0.6 ± 0.0</td>
<td>38 ± 8.1</td>
</tr>
<tr>
<td></td>
<td>2012</td>
<td>2.9 ± 1.5</td>
<td>0.3 ± 0.1</td>
<td>0.9 ± 0.3</td>
<td>79 ± 30</td>
</tr>
<tr>
<td></td>
<td>mean</td>
<td>2.1</td>
<td>0.2</td>
<td>0.8</td>
<td>58</td>
</tr>
<tr>
<td>April</td>
<td>2010</td>
<td>6.2 ± 0.9</td>
<td>0.0 ± 0.0</td>
<td>7.3 ± 1.9</td>
<td>10 ± 11</td>
</tr>
<tr>
<td></td>
<td>2011</td>
<td>3.7 ± 2.5</td>
<td>0.1 ± 0.0</td>
<td>0.8 ± 0.2</td>
<td>76 ± 49</td>
</tr>
<tr>
<td></td>
<td>mean</td>
<td>4.9</td>
<td>0.1</td>
<td>4.1</td>
<td>43</td>
</tr>
<tr>
<td>August</td>
<td>2010</td>
<td>1.4 ± 0.3</td>
<td>0.7 ± 0.2</td>
<td>0.8 ± 0.0</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>2011</td>
<td>1.3 ± 0.2</td>
<td>0.3 ± 0.2</td>
<td>0.6 ± 0.3</td>
<td>2.6 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>mean</td>
<td>1.3</td>
<td>0.5</td>
<td>0.7</td>
<td>1.7</td>
</tr>
</tbody>
</table>
Table 5. Nitrification and \( \text{NH}_4^+ \) regeneration rates. Rates listed are the mean and standard deviation for each sampling trip. Nd is for no data.

<table>
<thead>
<tr>
<th>Month</th>
<th>Year</th>
<th>( \text{NH}_4^+ ) Regeneration (nmol N L(^{-1}) h(^{-1}))</th>
<th>Nitrification (nmol N L(^{-1}) h(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>2011</td>
<td>17.2 ± 5.67</td>
<td>27.4 ± 8.21</td>
</tr>
<tr>
<td></td>
<td>2012</td>
<td>10.4 ± 0.48</td>
<td>15.8 ± 9.58</td>
</tr>
<tr>
<td>April</td>
<td>2010</td>
<td>4.85 ± 1.42</td>
<td>nd</td>
</tr>
<tr>
<td></td>
<td>2011</td>
<td>27.8 ± 1.18</td>
<td>24.9 ± 16.6</td>
</tr>
<tr>
<td>August</td>
<td>2010</td>
<td>12.5 ± 9.37</td>
<td>nd</td>
</tr>
<tr>
<td></td>
<td>2011</td>
<td>15.2 ± 3.11</td>
<td>1.02 ± 0.32</td>
</tr>
</tbody>
</table>
Table 6. Uptake rates of NH$_4^+$, NO$_3^-$, and urea in the western Arctic water column. Rates are reported as the mean with range in parentheses. Where there are no uptake values reported, that column was left blank. All values are for size fractions greater than 0.7 μm, with the exception of the studies by Kristiansen (Kristiansen et al. 1994; Kristiansen and Lund 1989), who used GF/C filters (nominal pore size = 1.2 μm) and (Simpson et al. 2013a), who corrected their rates for bacterial retention on the GF/F filter. Values determined by integration over the euphotic zone are denoted by $Z_e$ with mean depth in parentheses. SCM stands for subsurface chlorophyll max, with mean depth in parentheses. MIZ stands for marginal ice zone, which includes a mix of ice-free and ice-covered conditions.

<table>
<thead>
<tr>
<th>Region</th>
<th>Season</th>
<th>Year</th>
<th>Sampling depth</th>
<th>Ice cover?</th>
<th>NH$_4^+$ nmol N L$^{-1}$ h$^{-1}$</th>
<th>NO$_3^-$ nmol N L$^{-1}$ h$^{-1}$</th>
<th>Urea nmol N L$^{-1}$ h$^{-1}$</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baffin Bay</td>
<td>Aug - Sep</td>
<td>1978</td>
<td>$Z_e$ (34 m)</td>
<td>no</td>
<td>2.26 (0.87 - 5.86)</td>
<td>3.03 (1.05 - 7.18)</td>
<td></td>
<td>Harrison et al. 1982</td>
</tr>
<tr>
<td></td>
<td>Jul - Aug</td>
<td>1980</td>
<td>$Z_e$ (33 m)</td>
<td>no</td>
<td>2.62 (0.65 - 5.29)</td>
<td>1.30 (0.24 - 5.55)</td>
<td>1.02 (0.26 - 2.17)</td>
<td>Harrison et al. 1985</td>
</tr>
<tr>
<td></td>
<td>Sep</td>
<td>1999</td>
<td>100% light</td>
<td>no</td>
<td>6.88 (0.59 - 17.8)</td>
<td>6.06 (0.57 - 13.6)</td>
<td>1.66 (0.21 - 3.74)</td>
<td>Fouilland et al. 2007</td>
</tr>
<tr>
<td></td>
<td>Sep</td>
<td>1999</td>
<td>$Z_e$ (42 m)</td>
<td>no</td>
<td>10.95 (2.86 - 28.1)</td>
<td>12.62 (4.52 - 36.0)</td>
<td>4.29 (1.43 - 7.38)</td>
<td>Garneau et al. 2007</td>
</tr>
<tr>
<td></td>
<td>Sep$^1$</td>
<td>1999</td>
<td>$Z_e$ (45 m)</td>
<td>yes</td>
<td>2.22</td>
<td>3.56</td>
<td>1.11</td>
<td>Garneau et al. 2007</td>
</tr>
</tbody>
</table>

53
<table>
<thead>
<tr>
<th>Location</th>
<th>Season</th>
<th>Year(s)</th>
<th>Depth</th>
<th>Layer</th>
<th>Data</th>
<th>Organism</th>
<th>Surface</th>
<th>Bloom</th>
<th>Post-bloom</th>
<th>Pre- and post-bloom</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barents Sea</td>
<td>Summer</td>
<td>1984</td>
<td>unknown</td>
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<td>surface</td>
<td>MIZ</td>
<td>4.7</td>
<td>1.8</td>
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<tr>
<td></td>
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<td>(&lt;15 m)</td>
<td></td>
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<tr>
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<td>12.5</td>
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<tr>
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<td></td>
<td></td>
<td></td>
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<td>Aug – Oct</td>
<td>2005-2007</td>
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<td>no¹</td>
<td>0.84</td>
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</tr>
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<td>0.07</td>
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<tr>
<td></td>
<td>Bloom</td>
<td>1984-1988</td>
<td>Ze (50 m)</td>
<td>no</td>
<td>2.98</td>
<td>3.53</td>
<td>7.11</td>
<td></td>
<td></td>
<td></td>
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<td>Variance</td>
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Lee et al. 2010
Yun et al. 2012
Lee et al. 2007
Lee et al. 2012
this study
Keene et al. 1991
Smith 1993
Smith 1995
Lee et al. 20122
Lee et al. 20122
May – Aug 1993 100% light no 2.36 0.84 Smith et al. 1997
(0.16 – 7.24) (0.00 - 5.17)

May – July 1993 \( Z_t (< 70 \text{ m}) \) no 1.35\(^{(6)}\) 1.94\(^{(6)}\) Daly et al. 1999
(0.00 – 23.9) (0.00 – 108)

1 Garneau et al. make a distinction between early September and late September in their study, as the latter half of the month involved rapidly increasing ice cover at the experimental location.

2 Rate data provided by personal communication.

3 Of the 13 Beaufort Sea locations in the Martin et al. study, 3 had some sea ice present.

4 Normalized to under-ice conditions.

5 The 1989 cruise (Smith 1993) occurred during a \textit{Phaeocystis} bloom and is the most likely explanation for such high rates.

6 Daly et al. (1999) report median values.
Figure legends

Figure 1. Sampling location. Samples were taken approximately 2.5 km north of Barrow, Alaska indicated by the black circle. Depth contours are 50 m intervals. See Table 1 for exact locations and bottom depths.

Figure 2. Ambient nutrient comparisons. Values are only shown for the depths at which incubations were performed. The dashed line represents Redfield ratios of C:N:Si:P of 106:16:15:1. Error bars are standard deviation.

Figure 3. Absolute uptake rates of nitrogen. Mean rates of N uptake (two experiments for each of two years) for each substrate tested, with error bars representing the standard deviation (n = 4). Solid bars are >3.0 μm fraction, and hatched bars are GF/F – 3.0 μm fraction. The dashed line in the summer panel corresponds to the uppermost y-axis scale of the winter and spring panels, and is included solely to provide a reference.

Figure 4. Absolute uptake rates of carbon. Mean rates of C uptake for each substrate tested, with error bars representing the standard deviation. Solid bars are >3.0 μm fraction, and hatched bars are GF/F – 3.0 μm fraction. The dashed line in the summer panel corresponds to the uppermost y-axis scale of the winter and spring panels, and is included solely to provide a reference.

Figure 5. Ammonium cycling processes. Concentrations of NH₄⁺ plotted against rates of nitrification and NH₄⁺ regeneration. The regression lines are for all values in the plot (see text for significance and R² values).
Figure 1
Figure 3

[Graphs showing the concentration of ions and urea in winter, spring, and summer seasons.]
Figure 4
Figure 5

Regeneration (nmol L\(^{-1}\) h\(^{-1}\))

Nitrification (nmol L\(^{-1}\) h\(^{-1}\))

\[\text{NH}_4^+ \ (\text{umol L}^{-1})\]
Chapter 3 – Nitrogen uptake rates of low- and high-nucleic acid bacterial subgroups from the coastal Arctic (Chukchi Sea)

Author’s Note

The number of samples analyzed for this chapter was small due to limitations in access to a high-speed cell sorter. Additional samples will be processed on a newly acquired BD Influx cell sorter prior to submission of this chapter to a journal.
Abstract

In flow cytometric analyses of marine bacterioplankton, there is a bimodal distribution of subpopulations that are ubiquitous – one with low nucleic acid (LNA) and one with high-nucleic acid (HNA) content. In this study we used nitrogen (N) stable isotope tracer techniques and flow cytometric sorting to determine rates of N uptake of LNA and HNA populations collected in the shallow coastal environment of the Chukchi Sea during winter, spring and summer. To our knowledge this is the first report of N uptake in LNA and HNA populations. In summer, HNA cells comprised a higher percentage of total bacterial abundance compared to winter. These HNA cells had lower cellular N content than LNA cells, but had higher uptake rates of all substrates. Specific and absolute uptake rates of ammonium and an amino acid mixture were higher than for nitrate and urea in both groups. Seasonal differences in the relative abundance and N uptake rates of LNA and HNA fractions indicate that these groups are both active, but they appear to respond differently to environmental signals.


Introduction

In marine waters, phytoplankton and bacteria compete intensely for limited resources to fuel their metabolisms. Traditionally, it has been methodologically difficult to separate microbial populations such as phytoplankton and bacteria, and therefore challenging to ascribe biogeochemical rate measurements to these two major microbial groups. A promising approach is to combine flow cytometric analysis and sorting with \( ^{15}\text{N} \) stable isotopes techniques (Lipschultz 1995), which allows accurate and unambiguous determination of uptake of N compounds by different identifiable populations.

With a flow cytometer, large numbers of particles can be identified quickly and accurately based on size, density and fluorescent characteristics such as chlorophyll pigments (Gasol & del Giorgio 2000). With the development of a wide variety of fluorescent stains that bind to DNA, organisms that do not have any naturally fluorescent properties can be distinguished from detritus and other particles, leading to quick and accurate bacterial abundance measurements along with the ability to identify and sort cells for further analysis (reviewed in Lomas et al. 2011).

In flow cytometric analyses of aquatic systems, the bacterioplankton (which includes archaea, but will heretofore be referred to as "bacterial") fraction of aquatic samples clusters in two distinct groups, commonly referred to as low nucleic acid (LNA) and high nucleic acid (HNA) fractions (Li et al. 1995, Gasol et al. 1999, Lebaron et al. 2001). This remains true even with samples from a variety of locations and the use of different staining protocols (Bouvier et al. 2007, Wang et al. 2009). Although these

65
clusters are found universally, there are conflicting reports of their ecological significance or what they indicate about the populations themselves (Bouvier et al. 2007). Initially, HNA and LNA cells were generally considered as the active and the inactive fractions, respectively (Jellett et al. 1996, Gasol et al. 1999, Lebaron et al. 2001, Vaqué et al. 2001). Some studies investigating these two fractions found higher bacterial production in the HNA cells (Lebaron et al. 2001, Lebaron et al. 2002, Servais et al. 2003, Morán et al. 2007, Morán et al. 2011), but others found that the LNA fraction could be at least as active as the HNA fraction (Zubkov et al. 2001, Mary et al. 2006, Wang et al. 2009). There is also conflicting evidence of whether the LNA and HNA groups consist of distinct phylogenetic groups (Zubkov et al. 2001, Schattenhofer et al. 2011, Vila-Costa et al. 2012) as opposed to significant overlap between the two groups (Servais et al. 2003, Longnecker et al. 2005). Differences in results are possibly related to broad ecological parameters, as it has been found that LNA cells are more active in coastal regions relative to the oligotrophic open ocean (Servais et al. 2003, Longnecker et al. 2005, 2006).

Most studies of LNA and HNA populations have taken place in temperate regions, although a few studies have found that these groups are also present in the higher latitudes (Corzo et al. 2005, Belzile et al. 2008, Garzio et al. 2013, Schattenhofer et al. 2011). In Antarctica (Corzo et al. 2005) and the Beaufort Sea in the Arctic (Belzile et al. 2008), HNA cells had increased growth rates in response to the spring phytoplankton bloom, while the LNA cells growth rate was delayed by more than two months. These results imply that the HNA population has greater genetic plasticity and the ability to react more quickly to changing environmental conditions. There is no corresponding
information on LNA and HNA groups in the Chukchi Sea, even though this is an area of

Though the measurement of N uptake rates of the LNA and HNA subpopulations
could provide detailed empirical information on the activity of the prokaryotic
assemblage, no published studies exist. The use of $^{15}$N tracers in combination with flow
cytometric sorting was pioneered by Lipschultz (1995), using natural phytoplankton in
coastal Maine. Further work used this approach to measure inorganic N uptake by
phytoplankton in the Sargasso Sea (Casey et al. 2007, Fawcett et al. 2011) and to
determine phytoplankton-specific from bacterial-specific inorganic and organic N uptake
in the mid-Atlantic Bight (Bradley et al. 2010a), Chesapeake Bay (Bradley et al. 2010b),
and a mesocosm study in a Norwegian fjord (Bradley et al. 2010c). Similarly,
methionine with isotopically labeled sulfur has been used to investigate amino acid
uptake in Prochlorococcus (Zubkov et al. 2003, Zubkov & Tarran 2005). Here we use
flow cytometric sorting of LNA and HNA populations of the coastal Chukchi Sea to
determine N uptake rates within those two groups and to highlight potential seasonal
differences in these populations.

Materials and methods

Sampling

Water column samples were taken as part of a larger study investigating N uptake
and regeneration in the coastal Arctic; details of the sampling methodology can be found
in Chapter 2. Briefly, during April and August of 2010 and 2011 and January of 2011
and 2012, water samples were taken off the coast of Barrow, Alaska with a small, low-pressure pump from beneath the sea ice or a small boat, depending on conditions. Replicate subsamples were incubated with tracer (<10%) additions of $^{15}$N-labelled ammonium chloride (98.85% $^{15}$N), potassium nitrate (98%), urea (98%) or an amino acid mixture (96-99%; Cambridge Isotope Laboratories). The incubation bottles were placed in an environmental control chamber at ambient light and temperature for 24 hours.

**Nutrient analysis**

Nutrient analyses were performed on sub-samples after filtration and then frozen at -20°C until analysis. Concentrations of ammonium ($\text{NH}_4^+$) were measured in triplicate using the phenol-hypochlorite method (Koroleff 1983). Nitrate ($\text{NO}_3^-$) and nitrite ($\text{NO}_2^-$) concentrations were measured in duplicate on a Lachat QuikChem 8500 autoanalyzer (Parsons et al. 1984). Dissolved primary amines (DPA) were measured in triplicate on a Shimadzu RF-1501 spectrofluorometer following the o-phthaldialdehyde method (Parsons et al. 1984). Urea was measured in duplicate using the manual monoxime method (Price & Harrison 1987).

**Flow cytometric sorting**

At the end of the incubation, samples were concentrated over 0.2 μm Supor® polyethersulfone filters (Pall Corporation) under gentle vacuum pressure (<5 Hg). When a small volume (<30 mL) of sample remained, the filter was gently sparged using a clean pipette, and 10 mL of sample was placed in a 15 mL polypropylene tube. Immediately
before the filter went completely dry, it was removed, placed in the same tube, and gently agitated to recover cells from the filter (see Bradley et al. 2010b for full details on the method). Samples were fixed with an addition of 63 μL of paraformaldehyde (0.2% final concentration), allowed to sit for 10 minutes, and flash frozen in liquid nitrogen. Samples were stored at -80°C until analysis.

Flow-cytometric analyses were performed on a high-speed Influx cell sorter (BD Biosciences) with a blue-light (488 nm) laser operating at 200 mW and a bandpass filter at 531/40 nm (green fluorescence) and a longpass filter at 692/20 nm (red fluorescence). Staining with SYTO 13 green fluorescent nucleic acid stain in DMSO (1:100 final dilution of the commercial stock; Invitrogen) was carried out for 5 minutes in the dark at room temperature. The sample was passed through a 50 μm CellTrics® (Partec) to reduce any chance of clogging the nozzle and run with a sheath fluid of sterilized and 0.2 μm filtered phosphate buffered saline (PBS; Fisher Scientific). A 70 μm nozzle tip was used with a sample pressure of 28.5 PSI and sheath pressure between 29.5 and 30.0. Post-acquisition analysis was performed with FlowJo version X (Tree Star Inc.).

Cells were detected and gated according to relative values of side scatter (SSC) and green fluorescence (FL1), with greater FL1 indicating HNA populations (Gasol & del Giorgio 2000). The Influx sorter was set in “1.0 drop pure” sort mode and maintained a sorting rate of 800 – 1500 events s⁻¹, depending on the density of cells present. The populations were simultaneously sorted into several separate sterilized 0.5 ml polypropylene tubes. To acquire enough cells for isotopic and mass analysis, approximately the entire volume of sample (10 mL) was processed. The biggest
constraint of this study was the number of uptake rates we were able to measure based on
the access we had to the flow cytometer we used. Timing is an issue because it takes
considerable time to sort enough biomass to get a reliable measurement on the mass
spectrometer. An average sort time for each sample was close to four hours. This led to
the small number of samples able to be processed. After staining, LNA and HNA
populations were fairly distinct (Figure 1). Cell densities were high enough to reliably
sort at a target event rate of over 1000 events s\(^{-1}\).

Samples for each given sorted population were mixed and immediately filtered
through 0.2 µm silver membrane filters (Sterlitech Corporation). The filter tower was
rinsed with 0.2 µm filtered ultrapure water (18.2 M\(\Omega\); EMD Millipore) to ensure
complete capture of sorted populations. Filters were placed in cryovials and kept at -
20°C until analyzed on a Europa Geo 20/20 isotope ratio mass spectrometer with an
ANCA autosampler. A \(^{14}\)N carrier addition was made to ensure there was enough mass
for analysis. Carrier additions were estimated based on the number of cells sorted and a
N biomass conversion of 12 fg N cell\(^{-1}\) (Vrede et al. 2002).

Specific uptake rates (\(V; \text{h}^{-1}\)), which describe the physiological ability of cells to
assimilate N (Dugdale & Wilkerson 1986) were calculated as in Lipschultz (1995). We
calculated absolute uptake rates (\(\rho; \text{nmol N L}^{-1} \text{h}^{-1}\)) by multiplying the specific uptake
rate by the particulate nitrogen in the LNA or HNA bacteria:

\[
\rho_{HNA} = V \cdot [PN]_{HNA}
\]  

(1)

where \([PN]\) is the particulate N, estimated by the following equation:

\[
[PN]_{HNA} = BA \cdot \%HNA \cdot N_{cell}^{-1}
\]  

(2)
where BA is the bacterial abundance (cells L\(^{-1}\)) at the start of the incubation, %HNA is the percent of HNA cells determined with the flow cytometer, and N cell\(^{-1}\) is the mean mass of N present in a HNA cell, which was determined by dividing the mass of N of the sorted HNA cells by the number of HNA cells sorted.

**Results**

*Environmental conditions*

Conditions varied widely between the seasons, although the water column remained well mixed during all sampling, as fully explained in Chapter 2. Briefly, the water column in April had lower ice cover in 2010 than 2011 (0.55 m and 1.4 m thickness respectively). The water column was well mixed with regard to temperature (-1.8°C) and salinity (mean = 32). Photosynthetically active radiation (PAR) averaged 6.4 \(\mu\)mol quanta\(^{-1}\) m\(^{-2}\) s\(^{-1}\). Chlorophyll \(a\) (Chl \(a\)) was higher in 2011 (mean and standard deviation = 1.0 ± 0.52 \(\mu\)g L\(^{-1}\)) than 2010 (0.08 ± 0.03 \(\mu\)g L\(^{-1}\)), but bacterial abundance exhibited the opposite pattern, with 0.9 ± 0.06 x10\(^8\) cells L\(^{-1}\) in 2010 and 3.2 ± 0.9 x10\(^8\) cells L\(^{-1}\) in 2011. Ambient substrate concentrations were all below 1.0 \(\mu\)mol N L\(^{-1}\) (Table 1).

August conditions were ice-free and the water temperature increased to a mean of 5.5°C, while salinity decreased slightly (mean = 31). With the lack of ice cover and increased hours of daylight, PAR was much higher than the other seasons, at 55 \(\mu\)mol quanta\(^{-1}\) m\(^{-2}\) s\(^{-1}\). Chl \(a\) and bacterial abundance both reached their maximum values during summer. The multiyear mean of Chl \(a\) was 0.6 ± 0.2 \(\mu\)g L\(^{-1}\). Bacterial abundance was lower in 2010 (5.9 ± 3.8 x10\(^8\) cells L\(^{-1}\)) than 2011 (20 ± 14 x10\(^8\) cells L\(^{-1}\))

71
respectively. Ambient nutrient concentrations reached seasonal lows, with all measured compounds below 0.5 μmol N L\(^{-1}\) (Table 1).

January was characterized by moderate ice cover (0.9 m in 2011 and 2012), low temperature (-1.8°C) and PAR (0.2 μmol quanta\(^{-1}\) m\(^{-2}\) s\(^{-1}\)), with salinity (33) slightly higher than the other seasons. Chl \(a\) reached a seasonal low (0.02 ± 0.01 μg L\(^{-1}\)), but bacterial abundance was higher on average (2.9 ± 0.3 x10\(^8\) cells L\(^{-1}\)) than in April. Ambient NH\(_4^+\) and NO\(_3^-\) were high in January, but amino acid concentrations were slightly lower than in other seasons (Table 1).

**Characteristics of LNA and HNA clusters**

Clusters of LNA and HNA cells could be clearly separated based on their SSC and FL1 characteristics (Figure 1). The ratio of HNA cells to the total (%HNA) ranged from 32 – 91%, with the lowest values consistently found in January (mean ± standard deviation = 50 ± 15%) and the highest in August (78 ± 8.0%; Table 1). Cellular N content was greatest in the LNA fraction during April and August (Table 2). The same was true in January 2011, but in January 2012, however, HNA cells had twice the N content of the LNA cells.

**Nitrogen uptake**

The HNA fraction had higher NH\(_4^+\) specific uptake than the LNA fraction in all samples except in January 2012 (Table 1). This was more pronounced in the summer sample, when the specific uptake of NH\(_4^+\) in the HNA fraction was 94% greater than the
LNA fraction. In spring and winter, the HNA fraction had NH$_4^+$ specific uptake rates that were 64% and 53% greater than the LNA fraction, respectively. Similar results were found for absolute uptake of NH$_4^+$ – all of the samples had higher NH$_4^+$ absolute uptake in the HNA fraction. Likewise, both specific and absolute uptake of NO$_3^-$ was higher in the HNA fraction, regardless of season. The same holds true for urea, but only a summer sample was analyzed, so there is no comparison for winter or spring. Uptake of amino acids by the HNA fraction was higher in summer, but approximately equal during winter.

When considering NH$_4^+$ and AA uptake, there is no significant correlation between %HNA and specific or absolute uptake, although they appear to be weakly positive (data not shown). Even when using all of the substrates in the analysis and normalizing for the amount of uptake within each N compound, there is no significant relationship between the %HNA and the percent of specific or absolute uptake (Figure 2). In fact, none of the factors we tested (%HNA, seasons, ambient nutrient concentration) were significant predictors of N uptake, which may have been a function of the small sample size.

**Discussion**

In the Chukchi Sea, N uptake rates of phytoplankton have been determined by filtration through GF/F filters (nominal pore size = 0.7µm), but there has been no accounting for bacterial uptake (Lee et al. 2007, Lee et al. 2010). In order to distinguish bacterial uptake from phytoplankton uptake, it is necessarily to positively identify bacterial groups and determine their uptake rates unequivocally. Other Arctic studies
have attempted to specifically account for bacterial N uptake using size fractionation (Allen et al. 2002), but this technique has limited success due to the overlapping size of bacteria and phytoplankton and variable retention of bacteria on filters (Lee & Fuhrman 1987, Gasol & Morán 1999). Simpson et al. (2013) tried to overcome that limitation by mathematically accounting for bacteria passing through the GF/F filters and then caught on 0.2 \( \mu m \) silver filters. Metabolic inhibitors have also been used (Fouilland et al. 2007), but that technique is notoriously unreliable due to lack of specificity and incomplete inhibition (Oremland & Capone 1988). Thus, we used \( ^{15}N \) uptake after flow cytometric sorting to describe the activity of LNA and HNA fractions in coastal Arctic samples.

**Seasonal differences**

Abundance is not a good indicator of bacterial activity (Yager & Deming 1999, Alonso-Sáez et al. 2008). This also appears to be true within the bacterial nucleic acid groups. There was a difference in %HNA by season, but %HNA had a very weak correlation with different rates of uptake (\( R^2 = 0.16 \) for specific uptake and 0.01 for absolute uptake). In high latitude waters, it has been found that during winter HNA cells are preferentially grazed (Garzio et al., in press, Gasol et al. 1999, Corzo et al. 2005) and %HNA falls below 50% (Belzile et al. 2008). This seasonal difference appears to be unique to the polar regions. In temperate waters, %HNA was lower in summer compared to winter (Calvo-Díaz & Morán 2006, Morán & Calvo-Díaz 2009, Vila-Costa et al. 2012). It is thought that HNA is an indication of larger genomes, which allows bacterioplankton to be more adaptable to conditions and therefore grow faster in response to changing
environmental variables (Schattenhofer et al. 2011). If this is true, then it would imply that rather than any static measured nutrient or physical parameter, it is the rate of change in that parameter over time that is an important factor determining the activity of the LNA and HNA fractions. Consistent with this idea, Belzile et al. (2008) found that LNA had slower growth rates and a delayed response to the spring phytoplankton bloom in the coastal Beaufort Sea.

The difference between LNA and HNA in both specific and absolute uptake of N was greater during the late summer than winter. This would lend further support to the hypothesis that some feature of the ecosystem is allowing for greater relative activity of the LNA fraction during winter. It is possible that the lack of phytoplankton competition, along with static environmental conditions (e.g. light, temperature) favor the smaller LNA cells. Furthermore, there is little difference between overall bacterial abundance and the uptake rates during different seasons studied. It appears that these coastal Arctic bacterioplankton can maintain their N metabolism at consistent rates throughout the year.

**Substrate preference**

Each fraction effectively assimilated all of the N substrates tested. The LNA cells are clearly active, but not at the same rate as HNA cells (Table 1). Both LNA and HNA groups are actively taking up NH$_4^+$ and amino acids at higher rates than NO$_3^-$ and urea. The work done here was part of a larger study of N cycling in the Chukchi, which also found that NH$_4^+$ and amino acids are the preferred N forms (see Chapter 2). On the other hand, the difference between LNA and HNA uptake rates of all N compounds tested was
reduced during winter. This could be because there are different groups of bacteria that take up amino acids depending on the season (Alonso-Sáez et al. 2008, Nikrad et al. 2012).

It is known that bacteria can effectively compete with phytoplankton for inorganic N ($\text{NH}_4^+$ and $\text{NO}_3^-$) in the Arctic. During conditions of light limitation (i.e. depth and/or ice cover), bacteria assimilate $\text{NH}_4^+$ and $\text{NO}_3^-$ (Allen et al. 2002). In the Beaufort Sea, Simpson et al. (2013) found that bacteria were responsible for less than 15% of total N ($\text{NH}_4^+$, $\text{NO}_3^-$, and urea) uptake. On the other hand, Fouilland et al. (2007) found that bacteria could account for more than 50% of total $\text{NO}_3^-$ uptake. In this study, specific uptake of $\text{NO}_3^-$ was similar in magnitude to $\text{NH}_4^+$ specific uptake, which indicates that the bacteria have equal physiological capability to assimilate both forms of inorganic N.

**Conclusions**

This is the first report of N uptake by LNA and HNA populations of bacteria. We have shown that both fractions are actively assimilating N, including $\text{NO}_3^-$. We found little difference in uptake rates during different seasons even though the environmental conditions were drastically different. This was true despite the fact that HNA cells were relatively more abundant during late summer as opposed to winter. Further work is necessary to understand whether these are universal features of LNA and HNA bacteria, or whether the patterns we found are specific to this region.
Acknowledgments

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Li WKW, Jellett JF, Dickie PM (1995) DNA distributions in planktonic bacteria stained with TOTO or TO-PRO. Limnol Oceanogr 40:1485-1495


Tables

Table 1. N uptake rates. The ambient N concentration ([N]), percent of cells in the high nucleic acid cluster (%HNA), specific uptake and absolute uptake for high nucleic acid (HNA) and low nucleic acid (LNA) populations.

<table>
<thead>
<tr>
<th>Substrate added</th>
<th>Month</th>
<th>[N] (μmol N L⁻¹)</th>
<th>Bacterial Abundance (10⁸ cells L⁻¹)</th>
<th>%HNA</th>
<th>Specific Uptake (x10⁻⁴ h⁻¹)</th>
<th>Absolute Uptake (pmol N L⁻¹ h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH₄</td>
<td>April 2010</td>
<td>0.77</td>
<td>1.5</td>
<td>52</td>
<td>1.8</td>
<td>6.5</td>
</tr>
<tr>
<td></td>
<td>2011</td>
<td>0.81</td>
<td>2.6</td>
<td>73</td>
<td>12</td>
<td>13</td>
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<tr>
<td></td>
<td>August</td>
<td>2010</td>
<td>0.47</td>
<td>3.2</td>
<td>1.4</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>January</td>
<td>2011</td>
<td>1.9</td>
<td>2.7</td>
<td>49</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2012</td>
<td>0.55</td>
<td>2.7</td>
<td>46</td>
<td>12</td>
</tr>
<tr>
<td>NO₃</td>
<td>August</td>
<td>2010</td>
<td>0.20</td>
<td>3.2</td>
<td>74</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>January</td>
<td>2011</td>
<td>5.8</td>
<td>2.7</td>
<td>48</td>
<td>1.8</td>
</tr>
<tr>
<td>Urea</td>
<td>August</td>
<td>2010</td>
<td>0.18</td>
<td>3.2</td>
<td>70</td>
<td>1.0</td>
</tr>
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<td>Amino acids¹</td>
<td>August</td>
<td>2010</td>
<td>0.14</td>
<td>3.2</td>
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<tr>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td>2012</td>
<td>0.08</td>
<td>2.7</td>
<td>32</td>
<td>9.1</td>
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</tbody>
</table>

¹ Amino acid mixture consists of 20 amino acids labeled with ¹⁵N.
Table 2. Cellular N content. Calculated values of mean and standard deviation of N per cell for each sampling season. Lower case n is for number of samples.

<table>
<thead>
<tr>
<th>Month</th>
<th>Year</th>
<th>LNA</th>
<th>HNA</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>April</td>
<td>2010</td>
<td>55</td>
<td>36</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2011</td>
<td>116</td>
<td>67</td>
<td>1</td>
</tr>
<tr>
<td>August</td>
<td>2010</td>
<td>74 ± 60</td>
<td>15 ± 9</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>2011</td>
<td>56</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>January</td>
<td>2011</td>
<td>61 ± 49</td>
<td>28 ± 7</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>2012</td>
<td>29 ± 13</td>
<td>70 ± 26</td>
<td>3</td>
</tr>
</tbody>
</table>
Figures

Figure 1. Flow cytometry dot plot. Characteristic flow cytometry density plot and adjacent histogram of low- and high-nucleic acid content bacterioplankton, labeled as LNA and HNA respectively. Darker colors, along with higher peaks on the histograms, indicate greater density.
Figure 2. %HNA versus percent of uptake. Comparison of percent of high nucleic acid cells (%HNA) and the percent of specific (filled squares) and absolute (open squares) uptake in the HNA fraction. The dashed line represents 1:1 and is included solely for reference. Data include all substrates and seasons.
Chapter 4 – The effect of temperature on rates of ammonium uptake and nitrification in the western coastal Arctic Ocean during winter, spring, and summer
Abstract

Uptake and regeneration of ammonium (NH$_4^+$) play a key role in the structure and productivity of ecosystems. When nitrogen (N) is limiting, microbial community structure and function depend in part on whether NH$_4^+$ is assimilated directly into biomass or instead converted to nitrate (NO$_3^-$) via nitrification. It is not clear how these relative rates will change as the climate warms, especially at high latitudes. Here we present data on the sensitivity of NH$_4^+$ uptake and nitrification rates to short-term warming. Samples were collected from the Chukchi Sea off the coast of Barrow, Alaska during winter, spring, and summer and incubated for 24 hours in the dark with tracer-level additions of $^{15}$NH$_4^+$ at -1.5, 6, 13, and 20°C. Rates of NH$_4^+$ uptake and nitrification were measured in conjunction with bacterial production. In all seasons, NH$_4^+$ uptake rates were highest at 6 or 13°C, temperatures similar to current summertime conditions, but dropped off with increased warming, indicative of psychrophilic (i.e. cold-loving) microbial communities. In contrast, nitrification rates were less sensitive to temperature and were higher in winter and spring compared to summer. Contrary to expectations, a greater warming response ($Q_{10}$ in excess of 2) was observed under higher in situ N substrate concentrations. These findings suggest that as the Arctic coastal ecosystem continues to warm, NH$_4^+$ assimilation may become increasingly important relative to nitrification, potentially reducing the NO$_3^-$ available to primary producers.
1. Introduction

The Chukchi Sea receives significant nutrient inputs from the North Pacific Ocean [Codispoti et al., 2005], and its coastal shelves are regions of high nitrogen (N) turnover [Bates et al., 2005]. The western coastal Arctic is known for its high rates of denitrification [Devol et al., 1997] and overall N limitation of phytoplankton growth [Codispoti et al., 2009]. In the very shallow, nearshore area, there is a lack of established baseline data for N uptake rate measurements, especially during the dark and frigid winter months [Codispoti et al., 2005].

In the euphotic zone, light is thought to inhibit nitrification [Ward et al., 1984], and therefore diminish its importance, but recent evidence suggests this to be highly equivocal [Yool et al., 2007]. Even if nitrification is inhibited by light, it may depend on which wavelength [Guerrero and Jones, 1996] or whether the dominant taxa are archaeal or bacterial nitrifiers [Merbt et al., 2012]. Tremblay et al. [2008] hypothesized that winter nitrification, rather than physical processes could be the cause of consistent high NO$_3^-$ concentrations in the Arctic during winter. Although rates for pelagic nitrification in the Arctic are limited [Deal et al., 2011], there are numerous reports of nitrifying organisms in Arctic waters [Alonso-Sáez et al., 2012; Bano and Hollibaugh, 2000; Galand et al., 2009; Kalanetra et al., 2009]. The one study that has reported a seasonal comparison of nitrification measurements in coastal Arctic waters found much higher rates during winter than summer and attributed this to the chemoautotrophic potential of the prokaryotic community during the cold and dark conditions under sea ice [Christman et al., 2011].
There is an urgent need to understand N uptake and nitrification in the Arctic, as the region is warming faster than almost anywhere else on the planet [e.g. Serreze and Francis, 2006]. This warming has already caused reductions in sea ice extent and volume [Stroeve et al., 2007], freshening of the surface ocean [Yamamoto-Kawai et al., 2009], and numerous other impacts on the overall ecology of the system [Grebmeier, 2011; Wassmann et al., 2011]. Additionally, the nutrient regime of the Arctic is changing, with decreasing Pacific nutrient inputs [Codispoti et al., 2009] and increasing terrestrial runoff [Peterson et al., 2002]. Less well understood is how these large-scale physical changes will impact the biogeochemistry of the Arctic.

As the Arctic continues to warm and the ice-free season expands, our understanding of how the rates of NH$_4^+$ uptake and nitrification may change is not well constrained, especially during seasons other than summer. It is generally expected that rate processes respond positively to temperature increases, although a recent meta-analysis indicated that bacterial activity is not more sensitive to temperature increases in the polar regions as compared to the temperate zone; rather, the increased dissolved organic matter supply in the Arctic can explain most of the large increase in rates reported at low temperatures [Kirchman et al., 2009]. Synergy between temperature sensitivity and substrate concentration in Arctic marine systems has been reported for organic N compounds [Wiebe et al., 1992; Yager and Deming, 1999], but there is limited information for inorganic N substrates. In culture, there is evidence of NO$_3^-$ but not NH$_4^+$ uptake by psychrophiles being hampered at low temperatures [Reay et al., 1999]. Antarctic sea ice algae had maximum uptake rates of inorganic N between 0.5 and 3.0°C,
below which both NO$_3^-$ and NH$_4^+$ uptake decreased by at least half [Priscu et al., 1989].

In this study, we tested the sensitivity of NH$_4^+$ uptake and nitrification to warming in nearshore Chukchi Sea waters by incubating winter, spring, and summer seawater samples under a range of temperatures. This study provides a current baseline for these processes along with insight into how they may change under the specter of anthropogenic warming.

2. Materials and Methods

Sampling and analytical methods were part of a larger study (see Chapter 2) investigating overall N uptake and regeneration in the coastal Chukchi Sea near Barrow, Alaska. Briefly, coastal seawater was sampled during January, April, and August of 2011, and again during January of 2012. Experiments were performed multiple times during each trip, with the exception of the first winter, and each sampling was given a station identifier (SID; Table 1). All of the sampling was done at approximately the same location (71°21'N, 156°41'W) with a bottom depth of 17 m, except for the very sampling event (WIN3), when dangerous ice conditions forced us to move approximately 1 km northeast to a shallower site (71°21'N, 156°34'W; 6 m maximum depth).

Winter and spring samples were collected by traveling to near the outer edge of the fast ice by snow machine and then drilling through the ice to sample the seawater below. Summer samples were collected from a small boat. Once we accessed the water column, a low pressure electric bilge pump (Johnson Pump) was used to gently draw water through acid-washed and seawater-seasoned 1.25” i.d. Tygon® tubing (Saint
Gobain Performance Plastics) into 500 ml acid-washed PETG bottles, and then
inoculated with additions 0.2 µmol N L$^{-1}$ of $^{15}$N labeled ammonium chloride (98.85% $^{15}$N
NH$_4$Cl; Cambridge Isotope Laboratories) and 170 µmol C L$^{-1}$ $^{13}$C labeled sodium
bicarbonate (99% $^{13}$C NaHCO$_3$; Cambridge Isotope Laboratories). An additional set of
incubations was performed with 0.2 µmol N L$^{-1}$ of $^{15}$N labeled sodium nitrite (98% $^{15}$N
NaNO$_2$; Cambridge Isotope Laboratories) added. Though we did not know the ambient
concentrations at the time the incubations were set up, we used literature values to
estimate a target addition at or below 10% of ambient concentrations; the $^{15}$NH$_4^+$
additions averaged 18.2% over all seasons, while nitrite (NO$_2^-$) additions were essentially
100% due to the general lack of measurable NO$_2^-$ in the water column (see Results). To
prevent freezing during collection and transport back to the laboratory, the bottles were
placed in styrofoam freeze safes that were then filled with ambient seawater (see Table 1
for ambient water temperatures). The in situ mean water temperature in summer was
4.7°C so the lowest temperature incubation (i.e. -1.5°C) actually represents cooling of the
sample. Temperature changes during transport were limited to less than 0.3°C during all
seasons. Upon return to the lab, duplicate bottles were placed in dark water baths for 24
h at -1.5, 6, 13, and 20°C; digital thermometer probes were used to continuously monitor
incubation temperatures. At the end of the incubation, samples were filtered through
Whatman GF/F (0.7 µm nominal pore size) filters, which retained 61, 74, and 34% of the
bacterial cells during summer, spring, and winter respectively (see Chapter 2). The filters
were placed in cryovials and the filtrate into polypropylene tubes and frozen until
analysis.
Bacterial production was measured using the leucine incorporation method [Ducklow, 2000; Kirchman, 2001; Smith and Azam, 1992], and was measured on the initial sample and following each treatment by incubating triplicate 1.5 ml aliquots with tritiated leucine \((^{3}\text{H-leu}; \text{specific activity of 144 Ci mmol}^{-1})\) at a final concentration of 25 nM for 4 h in the dark. Incubations were terminated by adding 0.1 ml of 100% trichloroacetic acid (TCA) to each sample tube. Samples were centrifuged and protein was extracted by rinsing the samples again with 1 ml of ice-cold TCA and then by rinsing with 1 ml of 80% ethyl alcohol, with centrifugation between each rinse. After placement in a fume hood overnight to dry, 1 ml of UltimaGold™ scintillation cocktail (PerkinElmer) was added to each tube and the radioactivity was measured on a liquid scintillation counter. Killed controls were performed by adding the \(^{3}\text{H-leu}\) after killing the cells with the addition of TCA. Rates for the WIN1 experiment were anomalously inflated, but it is unclear why; \(Q_{10}\) results of that experiment are listed in Table 2, but not shown in Figure 3. During spring sampling, bacterial production from the same incubation bottles as the \(^{15}\text{N tracer}\) was only measured at one station (SPR1). In both cases (WIN1 and SPR1), we use the bacterial production results from a parallel set of incubations performed on the same water but without \(^{15}\text{N tracer}\) added. In August 2011, no radioisotope was available, but bacterial production rates were measured at the same site during August 2010, and are used here for comparison to the seasonal production rates. In all three instances, they are denoted by italics (Table 2) and dashed lines (Figure 3).
Bacterial abundance was measured on the initial water sample, prior to incubation, and then again following incubation of the different treatments. Triplicate 3.6 ml samples were fixed with 400 µl of formaldehyde and refrigerated at 6°C for 15 minutes to allow complete fixation. They were subsequently frozen at -80°C until analysis. After staining with SYBR Green (Invitrogen) and the addition of reference beads (Spherotech, Fluorescent Yellow Particles, 1.7-2.2 µm), samples were run in duplicate on a FACScalibur flow cytometer (Becton Dickinson) and analyzed using FlowJo software (Treestar Inc.).

Concentration of NH₄⁺ was measured in triplicate using the phenol-hypochlorite method [Koroleff, 1983]. Using a Lachat QuikChem 8500 autoanalyzer [Parsons et al., 1984], concentrations of NO₃⁻ and NO₂⁻ were measured in duplicate. All ¹⁵N and ¹³C uptake samples were run on a Europa GEO 20/20 mass spectrometer with an ANCA autosampler. We used the calculations of Dugdale and Goering [1967] and Hama et al. [1983] for N and carbon (C) uptake rates respectively. Solid phase extraction [Brzezinski, 1987; Dudek et al., 1986] was used to isolate the NH₄⁺ pool so that the NH₄⁺ atom % could be determined and used to correct for isotope dilution in calculations [Gilbert et al., 1982]. The denitrifier method [Sigman et al., 2001] was used to determine the atom% of the NO₃⁻ pool. Rates of nitrification were calculated by tracing the labeled N from NH₄⁺ into the NO₂⁻ and NO₃⁻ pools (NOₓ) collectively.

\[
\text{nitrification} = \frac{\text{atom\% } NO_3^-}{\text{atom\% } NH_4^+ \times \text{time}} \times [NO_3^-] \tag{1}
\]
The atom percent (atom%) of each substrate was corrected to atom% normal by subtraction of 0.3667. Time in this equation is the total time of the incubation and the NO\textsubscript{x} concentration is at the end of the incubation.

To assess the impact of temperature on the rates, \( Q_{10} \) values – which are a measure of the factor by which a biological rate is increased by a 10°C rise in temperature – were calculated for each positive change in rate at each temperature difference, by the following equation:

\[
Q_{10} = \left( \frac{\text{rate}_2}{\text{rate}_1} \right)^{\frac{10}{T_2 - T_1}}
\]  

[Segal, 1975] where the rate is the measured rate at a specific temperature, \( T \) is temperature of the incubation, and the subscripts refer to two distinct temperature incubations.

Data were analyzed using two-way analysis of variance (ANOVA) and Tukey's Honestly Significant Difference Method. Differences were considered significant at a p-value < 0.05. Due to the limited freezesafe space, we were only able to incubate a small number of replicates for each experiment.

3. Results

3.1. Ambient conditions

Water temperature during winter and spring was consistently -1.8°C. In summer of 2010, water temperature averaged 6.1°C, while in summer 2011, it was 4.7°C. Nutrient concentrations were highly variable from season to season and year to year in this extremely dynamic coastal environment. During January 2011, for example, the
ambient NH$_4^+$ concentration was three times higher than during January 2012 (Table 1). The seasonal minimum in both NH$_4^+$ and NO$_3^-$ concentrations occurred during the summer, and there was a much smaller range in NH$_4^+$ concentrations than NO$_3^-$ concentrations. The NO$_3^-$ concentrations during the summer were only 3% of those during winter and spring (Table 1). There was high variability in the concentration of these N compounds, even within a season, potentially indicating the influence of different water masses passing through the study area. In our study NO$_2^-$ was only detectable (limit = 0.03 µmol N L$^{-1}$) during one winter station (WIN3), and even then it was only 0.05 µmol N L$^{-1}$. As an intermediary in the nitrification process, the presence of NO$_2^-$ can indicate active net NH$_4^+$ oxidation. Its absence, however, can indicate tightly coupled NH$_4^+$ and NO$_2^-$ oxidation or an absence of nitrification altogether. We hypothesize that the low or undetectable NO$_2^-$ concentrations in this study indicate the former.

During January of 2011, bacterial abundance in the initial experimental samples from 2 m were $4.6 \pm 0.2 \times 10^8$ cells L$^{-1}$, whereas in January of 2012 abundances were lower ($2.5 - 2.9 \times 10^8$ cells L$^{-1}$; Table 1). During April, bacterial abundance was similar or slightly elevated ($2.1 - 3.9 \times 10^8$ cells L$^{-1}$) compared to January, three to eight times higher ($15 - 18 \times 10^8$ cells L$^{-1}$) in August.

3.2. Uptake of nitrogen and carbon

During January of 2011, when the ambient NH$_4^+$ concentration was three times higher, the uptake rate at in situ temperature (-1.5°C) was more than double that of 2012.
for all temperatures except 13°C during WIN3 (Figure 1). The response to warming was similarly enhanced by the higher NH$_4^+$ concentrations in 2011, with higher absolute rates, and a greater increase with warming. The rates peak at 6°C and plateau to a statistically equal value at 13°C, with a slight decrease at 20°C. Calculations of $Q_{10}$ (Table 2) reflect a strong sensitivity to temperature (3.1) from -1.5 to 6°C, and a $Q_{10}$ of 1 (indicating no additional temperature response) for 6 to 13°C. The January 2012 experiments (WIN2 and WIN3) both had maxima at 13°C with rates otherwise lower or equal among the other temperature ranges. The $Q_{10}$ values reflect this strong temperature sensitivity with a 6 to 13°C $Q_{10} > 4$ at WIN3, although WIN2 had a $Q_{10}$ of 2.

During spring, one of the stations (SPR3) had a strikingly similar pattern of NH$_4^+$ uptake to winter 2012 (WIN2 and WIN3), but at slightly reduced rates. Peaks in uptake rate were at 13°C, with a steep decline at 20°C, where the rate was less than those at -1.5 and 6°C. Uptake rates at SPR1 and SPR2 peaked at statistically equal rates in the 6 and 13°C incubations.

During the summer, uptake rates were significantly higher and the effect of warming was similar. The peak NH$_4^+$ uptake rate was measured at 13°C and was significantly different from -1.5°C ($p < 0.001$). The $Q_{10}$ values were above 2 for the -1.5 to 6°C increase during SUM2, but lower than 2 for the 6 to 13 and overall -1 to 13°C temperature increases. Again showing sensitivity to higher ambient NH$_4^+$ concentrations, both the absolute rates and $Q_{10}$ values were higher for all temperature increases during SUM1.
There was no increase in bacterial abundance or particulate N (data not shown) at the different temperature incubations. Cell-specific uptake rates of NH$_4^+$ (data not shown) had a similar relative pattern to the whole community rates, indicating that the change in rates is likely a physiological response, and not due to any short-term increases in biomass.

Ambient NO$_2^-$ was below our detection limit in all but a one set of samples (Table 1) and uptake rates were extremely low. The mean uptake rates at ambient temperature were 0.004 ± 0.002, 0.003 ± 0.000, and 0.043 ± 0.032 nmol L$^{-1}$ h$^{-1}$ for winter, spring, and summer, respectively. There was no discernible pattern to NO$_2^-$ uptake with changes in temperature, although the mean values for winter stations show a potential peak at 13°C, and the summer stations peak at either 13 or 20°C, but are not significantly different from the other temperatures.

Similarly, dark uptake of HCO$_3^-$ was low and only measurable during the summer. HCO$_3^-$ uptake displayed two maxima at the lowest and highest temperatures, with a minimum at the intermediate temperatures near in situ (4.7°C). Mean rates for the summer were 1.57 ± 0.46, 0.82 ± 0.18, 0.89 ± 0.07, and 1.58 ± 0.87 nmol C L$^{-1}$ h$^{-1}$ for the -1.5, 6, 13, and 20°C treatments, respectively. Since incubations were performed in the dark, HCO$_3^-$ uptake rates that we did find are likely due to a delay in the shutdown of the C fixation process.
3.3. Nitrification

During the ice-covered seasons of winter and spring, nitrification rates were much higher than NH$_4^+$ uptake rates. Nitrification rates had no statistically significant temperature maxima for any season (Figure 2). Overall, nitrification rates were two orders of magnitude higher during winter and spring than summer, and appear to be sensitive to high concentrations of NH$_4^+$, as WIN1 and SPR3 both had much higher relative rates that correlate to higher ambient NH$_4^+$ (Table 1). Consistent with a lack of effect, Q$_{10}$ values for nitrification were approximately 1 for all temperature differences during winter and spring (Table 2). Although one summer station (SUM1) had a slight peak at 13°C, and the Q$_{10}$ values are slightly higher for the lower two temperature ranges, the actual rate of nitrification during the summer was so small that any increase would likely have little impact on ecological processes. When we calculate the assimilation and nitrification rates over the time period of the incubation, there would still be enough NH$_4^+$ in the bottles to measure increased nitrification rates at all temperatures, and we conclude that there is no ceiling on the rate.

3.4. Bacterial production

Leucine incorporation rates followed a similar pattern as NH$_4^+$ uptake rates. During winter and spring, there is a peak at 13°C after which the rate falls, although the difference during spring is less pronounced (Figure 3). The Q$_{10}$ values for the -1.5 to 6°C range during winter and spring averaged 3.2 ± 0.5 and 2.7 ± 0.3 for each of those seasons, respectively (Table 2). The next temperature range (6 to 13°C), at an average Q$_{10}$ of 2.0
± 0.9 and 1.7 ± 0.6, were also not significantly different. At their respective ambient temperatures, bacterial production at station WIN3 was only slightly less than the spring mean. This is an interesting contrast to the results for the NH$_4^+$ uptake and nitrification, and may be due to sampling at a shallower site closer to the Beaufort Sea, which had higher dissolved organic C (DOC) concentrations. We found no stimulation of bacterial production compared to our controls that had no $^{15}$N substrates added (data not shown).

4. Discussion

Microbes have adapted to many extreme environments that seem to test the limits of life [D'Amico et al., 2006]. In extremely cold regions, the uptake of substrates at low temperatures (-1.5 to 4°C) can be significantly reduced due to decreased cell membrane fluidity [Nedwell, 1999]. To counteract this limitation, an increase in substrate concentrations can overcome the negative effect of low temperature on both mesophilic and psychrotolerant bacteria [Wiebe et al., 1992], although this effect is not always observed [Kirchman et al., 2005; Kirchman et al., 2009; Yager and Deming, 1999]. In this study, we sought to quantify the extent to which Arctic microplankton in their natural setting can utilize NH$_4^+$ over a seasonal cycle, and how they may respond to warming.

4.1. Ammonium uptake

The uptake of NH$_4^+$ displayed a distinct pattern of a prominent temperature effect, with an optimum at 13°C (Figure 1). Laboratory studies of pure cultures report microbes with low temperature optima, but found that NH$_4^+$ uptake was not temperature sensitive.
[Reay et al., 1999]. However, using a meta-analysis of environmental samples of dark
NH₄⁺ uptake, Smith and Harrison [1991] found Q₁₀ values greater than 2 for polar
regions. Additionally, Antarctic sea ice algae were found to have low temperature
maxima for N uptake and an extraordinarily high Q₁₀ of 15.7 for NH₄⁺ uptake between
2.0 and 3.0°C [Priscu et al., 1989]. Our results in the spring and summer consistently
show the greatest increase for the -1.5 to 6°C temperature change, with a range of Q₁₀
range of 1.4 - 3.2 (Table 2). During the winter, the same relationship holds in 2011, but
in the 2012 season, the maximum sensitivity was found between the 6 to 13°C
incubations.

While the community as a whole responds to increases in temperature, there is
also an underlying signal of an enhanced response to increased ambient NH₄⁺
concentrations, with a potential for synergistic responses if both factors change in the
future. Both the NH₄⁺ absolute uptake rates and the rate of increase with temperature (as
measured by Q₁₀) were positively correlated with higher NH₄⁺ concentrations at the
beginning of the incubations. Therefore additive effects of increased nutrient supply and
temperature should be considered when projecting future biogeochemical scenarios of the
coastal Arctic. These factors are already coupled, as temperature increases are reducing
ice extent and volume, which could lead to earlier increases in nutrient supply from the
receding ice edge, changing the timing of phytoplankton blooms, and increasing and
extending the time of dependence on regenerated production [Wassmann and Reigstad,
2011].
4.2. Nitrification

Consistent with other cold oceanic regions, nitrification rates were much higher than NH$_4^+$ uptake rates during the ice-covered seasons of winter and spring. In the North Sea over a winter cycle, Veuger et al. [2013] found that nitrification accounted for 89% of NH$_4^+$ sinks (defined as nitrification and NH$_4^+$ assimilation), which was much higher than rates from temperate areas where nitrification and assimilation were measured simultaneously [Lipschultz et al., 1986; Ward, 2005]. At our site, winter and spring nitrification was even more dominant, accounting for 99% of NH$_4^+$ consumption. This relationship holds even though there was a large difference in the year-to-year rate measured in winter at this study site. These differences in rates are likely due to higher NH$_4^+$ concentrations during 2011 (see below). In fact, even our highest measured rates are on the low end of a North Sea winter experiment, where the conditions included higher temperatures and NH$_4^+$ concentrations than our study, and measured nitrification rates ranging 41 – 221 nmol N L$^{-1}$ h$^{-1}$ [Veuger et al., 2013]. Similarly, our summer rate measurements are lower than nitrification rates measured in the perennially cold waters of the Southern Ocean [Bianchi et al., 1997]. During summer, the situation reverses, with NH$_4^+$ assimilation representing 91% of consumption processes. While we found nitrification rates that were exceedingly low and unchanging with temperature, NH$_4^+$ uptake is significantly higher (p < 0.05) during the summer compared to the other seasons.

Psychrophiles are known to nitrify at rates comparable to temperate mesophiles, but strong relationships between nitrification and environmental or ecological factors in the water column have yet to be established [Ward, 2008]. Phytoplankton can
outcompete nitrifiers for NH$_4^+$ in well-lit ocean layers [Ward, 2005], and that is likely the case during the polar summer. During the winter, when primary production is light limited, there is reduced competition from bacterial and archaeal N demand. In addition to light, temperature is often suggested to be a controlling environmental factor, at least for organic N [Pomeroy et al., 1990; Pomeroy et al., 1991; Wiebe et al., 1992]. Conversely, polar microorganisms are generally cold-adapted and polar bacteria only sometimes exhibit sensitivity to organic substrate availability as temperature decreases [Yager and Deming, 1999]. Uptake of inorganic N may exhibit a similar sensitivity. A recent set of experiments in the Puget Sound where incubations found no change in nitrification rates with the different temperature incubations ranging from 8 – 20°C [Horak et al., accepted]. In the Southern Ocean, Bianchi et al. [1997] also found no correlation between nitrification rate and temperature nor ambient NH$_4^+$ concentrations. Our results confirm that N uptake at low substrate concentrations is less sensitive to warming than when substrate concentrations are higher. This result may indicate that the more cold-tolerant members of polar microbial communities require higher substrate [e.g. Wiebe et al., 1992], whereas the true psychrophiles are less sensitive to substrate concentrations [Yager and Deming, 1999].

While there are no other studies that we know of testing the sensitivity of nitrification to temperature in polar waters, one ammonia oxidizer capable of growth down to -5°C has been isolated from southern Alaskan waters (sub-Arctic Pacific) but even when acclimated to low temperatures, this organism had an optimum temperature for nitrification of 22°C [Jones et al., 1988]. Nitrification rates have been subjected to
warming experiments in Arctic marine sediments and terrestrial soils, the latter of which are warming at an alarming rate. In Arctic marine sediments, optimum temperatures for nitrification are very low and rates decrease markedly when subjected to experimental warming [Thamdrup and Fleischer, 1998]. In Arctic soils, nitrifiers only responded to temperature above 10°C [Nadelhofer et al., 1991], at which point there was a two-fold increase in N mineralization. Avrahami and Conrad [2003] found subtle changes in bacterial community structure with increases in temperature. Much like our study, these temperatures are above the current normal range, but could portend a future in which nitrifier community shifts trigger rapid changes in NH$_4^+$ assimilation rates.

The microbial community itself is known to change over the seasonal cycle. Bacterial nitrifiers have been found during summer in Arctic surface waters [Hollibaugh et al., 2002]. During the Arctic winter, there is high crenarchaeal abundance [Alonso-Sáez et al., 2008] and these organisms are known nitrifiers both in the Arctic Ocean [Christman et al., 2011] and in other oceanic realms [Wuchter et al., 2006]. Crenarchaeota have been found with high affinities for NH$_4^+$ at both low [Martens-Habbema et al., 2009] and high [Morris et al., 2010] substrate concentrations, and have even recently been shown to use urea as both a C source and substrate for nitrification in the Arctic [Alonso-Sáez et al., 2012]. It has been proposed that Crenarchaea are responsible for high winter rates seen in this region [Christman et al., 2011], but this supposition has not been proven, as Kalanetra et al. [2009] found no genetic mechanism for nitrification (i.e. it lacked an ammonium monooxygenase gene) in central Arctic archaea, and abundance was not related to activity in another study of nitrification in high
latitude marine waters [Veuger et al., 2013]. On the other hand, Christman et al. [2011] found ammonium monooxygenase (which encodes for the enzyme responsible for the first step in nitrification process) rRNA copy numbers to be positively correlated with both the winter season and increasing NH$_4^+$ concentrations. While not tested directly in our study, the artificial lifting of these inhibitory or competitive conditions through dark incubations did not increase the rates, indicating that the native coastal bacterial community is sufficiently different during the summer season to respond. Another possible explanation is simply the difference in sampling location between the coastal and central Arctic, which may have distinct populations and metabolic capabilities [Kirchman et al., 2007].

4.3. Bacterial production

It has been proposed that bacterial production in the polar regions depends more on DOM supply than temperature [Kirchman et al., 2009]. In this experiment, where we artificially warmed the incubations on short time scales, the strongest bacterial response occurred in the first step warmer from ambient temperature (i.e. -1.5 to 6°C during winter/spring, and 6 to 13°C during summer), while the optimum temperature was 13°C for winter and spring. The bacterial community is therefore cold-loving or psychrophilic by traditional definitions [Morita, 1966; 1975]. Production increases were more pronounced in the winter of 2011 and the shallow site sampled in winter 2012 (WIN3). DOC was higher during winter than spring, and the highest concentration outside of summer was measured at WIN3, which could provide an explanation for the heightened
production during winter. A companion study performed during the summer of the prior year found a consistent rise in bacterial production with temperature, with an optimum at 20°C [Connelly et al., in preparation-a], which is the same relative increase from ambient temperature as the winter and spring incubations in our study (Figure 3). It is clear that the microbial community reacts to temperature increases the same way year-round while DOC concentrations are also potentially influential.

It is not surprising that we were unable to quantify C fixation during the winter and spring periods, as autotrophic production would ostensibly be very limited in the absence of light. On the other hand, chemoautotrophic organisms are likely present under sea ice, and one study reports HCO$_3^-$ uptake during the dark Arctic winter [Alonso-Sáez et al., 2010]. The fact that we did not observe dark C fixation in our study could indicate its absence or could be due to bacterial cells passing through the GF/F filters or that we did not add enough $^{13}$C label to discern a signal. Evidence for the latter is found in a companion study [Connelly et al., in preparation-b] where HCO$_3^-$ uptake was only measurable during winter in incubations with greater concentration of labeled HCO$_3^-$ and termination on smaller pore size filters (0.2 μm).

5. Conclusions

The future of the Arctic marine ecosystem is unknown, but the region is certainly changing. Rising air and water temperatures have already been recorded, along with subsequent losses in sea ice volume [e.g. Stroeve et al., 2012] While there is some evidence that chlorophyll $a$ in the coastal Arctic has been declining during the past
century [Boyce et al., 2010], it is expected that there will be increased annual phytoplankton production [Arrigo et al., 2008] and associated increased importance of DOC and bacterial activity [Kirchman et al., 2009]. In Arctic freshwater systems, higher bacterial and viral activity have already been observed [Söwström et al., 2007], and the marine system could be expected to follow the same trend. Based on the results of our study, we would expect further increases in NH$_4^+$ uptake during each season in the future, especially if the region warms faster during the summer months than winter and spring [Wang et al., 2012]. As light limitation is lifted earlier in the spring [Maslanik et al., 2007], nitrification processes will be at an even further disadvantage, driving the system closer to dependence on NH$_4^+$ supplies, rather than any NO$_3^-$ built up in the system during the ice-covered seasons. We have shown a large disparity in the rate of nitrification during the seasons sampled, with a potential dependence on nutrient supply. Even though we tested a wide range of temperatures, no changes were seen in the nitrification rates. Uptake of NH$_4^+$ on the other hand is highly sensitive to warming and our results suggest that the biogeochemistry and ecology of the western coastal Arctic will be impacted in the coming decades as the region warms.

Acknowledgments

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## Tables
Table 1. Chemical and biological parameters. Ambient water temperature, and nutrient and plankton concentrations at the time of water collection for each experiment and season. The station ID (SID) is as defined in the text. “Depth” is the depth sample taken. BDL is for below detection limit (0.03 µM).

<table>
<thead>
<tr>
<th>Date</th>
<th>SID</th>
<th>Depth (m)</th>
<th>Temp. (°C)</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>DOC (µmol C L$^{-1}$)</th>
<th>Chl a (µg L$^{-1}$)</th>
<th>Abundance (10$^8$ cells L$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jan 30</td>
<td>WIN1</td>
<td>2</td>
<td>-1.9</td>
<td>3.07</td>
<td>BDL</td>
<td>7.66</td>
<td>74.0</td>
<td>0.03</td>
<td>4.6 ± 0.2</td>
</tr>
<tr>
<td>Apr 26</td>
<td>SPR1</td>
<td>6.5</td>
<td>-1.6</td>
<td>0.81</td>
<td>BDL</td>
<td>8.41</td>
<td>67.9</td>
<td>0.11</td>
<td>2.6 ± 0.1</td>
</tr>
<tr>
<td>Apr 28</td>
<td>SPR2</td>
<td>6.5</td>
<td>-1.6</td>
<td>0.51</td>
<td>BDL</td>
<td>8.57</td>
<td>67.6</td>
<td>0.10</td>
<td>2.1 ± 0.2</td>
</tr>
<tr>
<td>Apr 30</td>
<td>SPR3</td>
<td>4</td>
<td>-1.8</td>
<td>1.25</td>
<td>BDL</td>
<td>11.4</td>
<td>67.2</td>
<td>0.06</td>
<td>3.9 ± 0.5</td>
</tr>
<tr>
<td>Aug 17</td>
<td>SUM1</td>
<td>4</td>
<td>+4.7</td>
<td>0.59</td>
<td>BDL</td>
<td>0.32</td>
<td>93.8</td>
<td>0.37</td>
<td>18 ± 0.5</td>
</tr>
<tr>
<td>Aug 18</td>
<td>SUM2</td>
<td>2</td>
<td>+4.7</td>
<td>0.47</td>
<td>BDL</td>
<td>0.33</td>
<td>93.7</td>
<td>0.62</td>
<td>15 ± 0.3</td>
</tr>
</tbody>
</table>

2012

<table>
<thead>
<tr>
<th>Date</th>
<th>SID</th>
<th>Depth (m)</th>
<th>Temp. (°C)</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>DOC (µmol C L$^{-1}$)</th>
<th>Chl a (µg L$^{-1}$)</th>
<th>Abundance (10$^8$ cells L$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jan 16</td>
<td>WIN2</td>
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<td>-1.8</td>
<td>0.60</td>
<td>BDL</td>
<td>11.7</td>
<td>82.3</td>
<td>0.03</td>
<td>2.5 ± 0.1</td>
</tr>
<tr>
<td>Jan 19</td>
<td>WIN3</td>
<td>1</td>
<td>-1.8</td>
<td>0.96</td>
<td>0.05</td>
<td>9.86</td>
<td>85.7</td>
<td>0.01</td>
<td>2.9 ± 0.5</td>
</tr>
</tbody>
</table>
Table 2. \( Q_{10} \) values. \( Q_{10} \) values for \( \text{NH}_4^+ \) uptake, nitrification, and bacterial production rates for each experiment. Values do not include the 20°C treatment, as all experiments had equal or decreased rates at that temperature. The station ID (SID) is as described in the text. Bacterial production \( Q_{10} \) data in italics for SUM1, SPR1, and parenthetically for WIN1 are from separate experiments performed during the same season, as explained in the Methods. n.d. is for no data.

<table>
<thead>
<tr>
<th></th>
<th>2011</th>
<th>2012</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>SPR1</td>
<td>SPR2</td>
</tr>
<tr>
<td>NH(_4^+) uptake</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-1.5 to 6°C</td>
<td>3.0</td>
<td>2.2</td>
</tr>
<tr>
<td>6 to 13</td>
<td>2.1</td>
<td>1.0</td>
</tr>
<tr>
<td>-1.5 to 13</td>
<td>2.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Nitrification</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-1.5 to 6°C</td>
<td>0.9</td>
<td>1.1</td>
</tr>
<tr>
<td>6 to 13</td>
<td>1.1</td>
<td>1.0</td>
</tr>
<tr>
<td>-1.5 to 13</td>
<td>1.0</td>
<td>1.1</td>
</tr>
<tr>
<td>Bacterial production</td>
<td></td>
<td></td>
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<tr>
<td>-1.5 to 6°C</td>
<td>2.5</td>
<td>n.d.</td>
</tr>
<tr>
<td>6 to 13</td>
<td>2.1</td>
<td>n.d.</td>
</tr>
<tr>
<td>-1.5 to 13</td>
<td>2.2</td>
<td>n.d.</td>
</tr>
</tbody>
</table>
Figures

Figure 1. Ammonium uptake rates. The station IDs for each experiment, as defined in the text and Table 1, are given next to each plot. Error bars are standard deviation. Note different y-axis scales. The SUM1 -1.5°C treatment was not corrected for isotope dilution, as indicated in the dashed line.
Figure 2. Nitrification rates. The station IDs for each experiment, as defined in the text and Table 1, are given next to each plot. SUM1 has no data for the -1.5°C treatment, as explained in the results section. Note that the y-axis scale for the summer plot is two orders of magnitude lower than the winter and spring. Error bars are standard deviation.
Figure 3. Bacterial production rates. The station IDs for each experiment, as defined in the text and Table 1, are given next to each plot. Error bars are standard deviation. Plots with dashed lines are for bacterial production experiments performed in separate incubations (see methods section), and are not labeled with an SID. Note the different y-axis scale for summer. Error bars are standard deviation.
Chapter 5 – Nitrogen uptake dynamics in landfast sea-ice of the Chukchi Sea
Abstract

In the Arctic, multiyear ice is likely being replaced by seasonal first year ice, and changes in community composition and biogeochemical cycling are projected to occur as a result. The coastal Chukchi Sea has a particularly productive landfast ice ecosystem, which could be a model for future scenarios of ice ecology throughout the Arctic, but is currently lacking direct measurements of nitrogen (N) cycling. Using stable isotopic tracers, we measured uptake and regeneration of ammonium ($\text{NH}_4^+$), nitrate ($\text{NO}_3^-$), and bicarbonate ($\text{HCO}_3^-$) along with incorporation of tritiated leucine at three depths within landfast sea ice in the Chukchi Sea near Barrow, Alaska during April 2011 and January 2012. Both inorganic and organic nutrient concentrations in the ice were generally higher than the water column, with the exception of phosphate ($\text{PO}_4^{3-}$), which may limit production in certain sections of ice. Primary production per liter at all ice depths was higher than in the water column during April, but was below the detection limit in January. Bacterial production in the bottom ice was consistently higher than the water column across seasons. Absolute uptake of $\text{NH}_4^+$ was highest in the bottom ice (0-10 cm from the ice-water interface), and higher than absolute uptake of $\text{NO}_3^-$ at all depth horizons except upper ice during January (30-40 cm). While N uptake rates were higher than the water column, nitrification rates were lower. Regeneration of $\text{NH}_4^+$ and $\text{NO}_3^-$ far exceeded uptake of those compounds. The high N uptake and regeneration rates in landfast ice, especially compared to the water column, highlights the importance of the biological sea ice community, and could provide an indication of how the biogeochemistry of the region will change in the future.
Introduction

The marine cryosphere is a critical driver of the Arctic ecosystem, with an established and active microbial community (Junge et al. 2004; Stoecker et al. 2000) despite the extreme seasonal fluctuations of light, temperature, and nutrient regimes within the ice. Primary production of the algae on the underside of sea ice seeds pervasive ice edge blooms (Brown and Arrigo 2013; Perrette et al. 2011) and provides a large seasonal pulse of primary production (Arrigo et al. 2010; Dupont 2012) that is a critical food source for heterotrophic protists (Riedel et al. 2007a) and pelagic zooplankton (Brown and Belt 2012a), thus supporting the short food webs in the Arctic (Hobson and Welch 1992). Organic matter decomposition from the receding ice during spring is an important mechanism for the supply of surface nutrients, which support regenerative production in the water column (Conover et al. 1999; Cota et al. 1990). Whatever organic matter escapes zooplankton and microbial heterotrophy in the water column ultimately sinks out to provide nutritional inputs to the benthos (Brown and Belt 2012b; Grebmeier et al. 2006; Renaud et al. 2007; Tremblay et al. 1989).

Sea ice organisms must deal with low temperatures and high salinity (Eicken 2003; Jones et al. 2012) and quickly adjust to changing light, temperature, and nutrient conditions (Krembs et al. 2011; Krembs et al. 2000). The sympagic (i.e. ice associated) community, along with physical mechanisms and characteristics such as freezing and brine drainage, along with ice age, help determine the chemical environment of the ice through biological processes like uptake and regeneration of nutrients. While there is low
primary and secondary productivity during dark winter months (Forest et al. 2011), ice algae still represent 17 to more than 25% of total Arctic primary production on an annual basis (Gosselin et al. 1997; Loose et al. 2011), and a vigorous ice bacterial community that is presumed to rapidly remineralize organic compounds (Arrigo and Thomas 2004; Harrison et al. 1990; Sala et al. 2010), thus providing a robust feedback and supporting greater biomass than would be expected in such an extreme environment. Even during the dark winter months, microorganisms remain active (Junge et al. 2004) although there is evidence that some cells merely survive due to lack of predation (Collins et al. 2010). As light limitation is lifted in the spring, bottom ice algae thrive by taking advantage of high nutrient surface waters and relatively high irradiance (Lee et al. 2008; Rózańska et al. 2009), resulting in the formation of blooms and the rapid drawdown of inorganic nutrients (Cota et al. 1990; Smith et al. 1997).

While the effects of nutrient concentrations and light limitation on primary production in sea ice have been studied, the actual rates of N uptake and regeneration has received relatively less focus, especially in overall ice profiles and during winter (Mock and Gradinger 1999; Mock and Thomas 2005). There are only a limited number of reports of N uptake in Arctic sea ice, and those that do exist are spread out in both space and time (Harrison et al. 1990; Krell et al. 2003). The Arctic is highly variable over both of these scales. The Chukchi Sea in particular is a unique environment with high Pacific nutrient inputs, tight pelagic-benthic coupling, and a relatively large indigenous human population that relies on traditional whaling and is therefore directly connected to the food web of coastal northern Alaska (Moore and Huntington 2008).
With high nutrient concentrations uncoupled from changes in algal biomass, it has been hypothesized that nutrient regeneration must be quite active in sea ice (Arrigo and Thomas 2004; Gradinger and Ikävalko 1998; Thomas et al. 1995). Regeneration of ammonium (NH$_4^+$) is especially important, as it potentially reduces the flux of nutrients to the benthos and is made available to ice-algae. Additionally, the different ecology of first year compared to multiyear ice may become more relevant. As climate warming causes rapid changes in the sea ice landscape, the Arctic is predicted to become seasonally ice free by 2080 or earlier (e.g. Boé et al. 2009; Serreze and Francis 2006; Stroeve et al. 2007; Stroeve et al. 2012).

In this study we quantified primary production, nutrient uptake and N regeneration in natural sea ice communities using tracer-level stable isotope additions of NH$_4^+$, nitrate (NO$_3^-$), and bicarbonate (HCO$_3^-$), along with bacterial production via tritiated leucine ($^3$H-Leu). Here we present the first data of N uptake and regeneration rates in landfast Arctic sea ice during spring and winter. These processes are especially important in the highly productive coastal regions where first year landfast ice can support higher sympagic biomass (Gosselin et al. 1997).

Materials and Methods

Sampling

On April 30, 2011 and January 19, 2012, a Kovacs Mark V coring system was used to retrieve multiple 14 cm diameter ice cores approximately 2.5 km off the coast of Barrow, Alaska (see Chapter 2 for a map and further description of the study site).
sampling site was on landfast sea ice overlying 17 m of water, within 50 m of a pressure ridge that prevented further access offshore. Cores were taken in a small (<10 m$^2$) area to minimize variability, with great care taken to avoid trampling the surface. Two initial cores were used to determine temperature and salinity. For temperature, a small hole was drilled into the ice every 5 cm and a digital probe inserted. Salinity measurements were obtained by slicing 10 cm sections of ice and each was placed in a plastic bag. After melting, salinity was determined by a single-operator multiparameter metabolic analyzer-coulometry system (Johnson et al. 1987). Brine channel volume was subsequently calculated by the equation of Frankenstein and Garner (1967) and brine salinity by the equation of Cox and Weeks (1983). Four additional cores were taken, each placed on an acid washed HDPE cutting board and sliced with a stainless steel saw to obtain replicates of three depths each: 0-10, 15-25, and 30-40 cm up from the ice-water interface, heretofore referred to as bottom, middle, and upper sections of ice respectively. Each of these sections were transferred to salt water seasoned and acid washed 4 L polycarbonate jars, mixed with equal volumes of 0.2 μm filtered artificial seawater (ASW) with a salinity of 35 to minimize osmotic shock (Garrison and Buck 1986). They were subsequently melted in a dark 5°C water bath over 24 hours.

Upon complete melting, corresponding sections from two cores were combined for a total of two replicates per substrate. Each sample was gently mixed and 2 L was transferred to PETG bottles for tracer incubations, while the remainder was used for ambient measurements of Chl $\alpha$, bacterial abundance, nutrient concentrations, and
bacterial production measurements, as outlined below. These ambient parameters were adjusted for dilution with added ASW by the following equation:

$$C_{ice} = \frac{(C_{tot} \cdot V_{tot} - C_{sw} \cdot V_{sw})}{V_{ice}}$$

where $C$ is substrate concentration and $V$ is sample volume of ice (ice), filtered seawater (sw), and the total mixture (tot), respectively. The $C_{ice}$ value was then multiplied by the brine volume fraction to get values of brine-normalized concentrations.

**Biomass, abundance, and production**

Concentrations of chlorophyll a (Chl a) were measured fluorometrically on sub-samples from the melted sections after extraction in 90% acetone on a Turner Design Model 10-AU fluorometer (Parsons 1984). Phaeophytin was subsequently measured on the same instrument after addition of hydrochloric acid. For bacterial abundance, triplicate whole water samples were fixed in the field with 0.5% additions of formaldehyde, stored for 15 minutes at 5°C to ensure complete fixation, and stored at -80°C until bacterial abundance analysis. Each sample was run in duplicate on a FACScalibur flow cytometer after staining with SYBR Green (Life Technologies) and the addition of 2.0 μm reference beads (Spherotech), and then analyzed using FlowJo cytometry analysis software (Treestar).

Bacterial community composition was determined by catalyzed reporter deposition fluorescence in situ hybridization (CARD-FISH; Pernthaler et al. 2002). Duplicate 15 mL water samples were fixed with 0.75 mL of 0.22 μm filtered formaldehyde and fixed at 4-5°C for 24 hours. Samples were gently (<5 mm Hg) filtered
onto an Isopore™ polycarbonate filter (Millipore) using a cellulose nitrate filter (Whatman) as a backer. After being allowed to dry for 5 minutes, the filters were placed in a plastic scintillation vial and stored at -80°C until analysis. Cells were embedded in 0.1% MetaPhor™ agarose (Lonza), dried at 35°C for 15 minutes, and then removed after the addition of ethanol. Filters were incubated in 2 mL lysozyme solution (Sigma-Aldrich) for 60 minutes at 37°C (for archaea, this was followed directly with achromopeptidase for 30 minutes at 37°C), and then washed in alternated baths of 95% ethanol and Milli-Q water. Cut sections of each filter were placed in a mixture of hybridization buffer and oligonucleotide probes (Table 1) and placed on a rotation shaker at 10 rpm at 35°C for 12 hours. After hybridization, each filter section was placed in 2 mL washing buffer and incubated at 37°C for 40 minutes. Amplification reagent (Perkin Elmer) was added, incubated at room temperature in the dark for 5 minutes, washed in PBS for 30 minutes at room temperature in the dark, rinsed briefly in Milli-Q water, then in ethanol, and air dried. Filter sections were mounted on a slide in a 4:1 mix of Citifluor (Citifluor Ltd) and Vecta Shield (Vector Laboratories, Inc.) containing 1 μg/mL of DAPI.

Bacterial production incubations were performed prior to 15N additions by incubating triplicate 1.5 mL aliquots with 3H-leu (specific activity of 144 Ci per mmol) at a final concentration of 25 nM for 4 hours in the dark. Incubations were terminated by adding 0.1 mL of 100% trichloroacetic acid (TCA) to each sample tube. Protein was extracted by rinsing the samples again with 1 mL of ice-cold TCA and then by rinsing with 1 mL of 80% ethyl alcohol, with centrifugation between each rinse. After drying overnight, 1 mL of scintillation cocktail (UltimaGold) was added to each tube and the
Radioactivity was measured on a liquid scintillation counter. Killed controls were performed by adding the $^3$H-leu only after killing the cells with the addition of TCA. No $^3$H-leucine incorporation was detected in the added ASW. To convert to units of carbon, we used a conversion factor of 1.5 kg C per mole of incorporated leucine, which is an empirically derived mean for the water column in the western Arctic (Kirchman et al. 2009).

**Nutrient concentrations**

Nutrient analysis was performed on sub-samples from the melted sections taken before the addition of labeled substrates (ambient values) after filtration by GF/F filters (0.7 μm nominal pore size; Whatman), and then frozen at -20°C until analysis. Concentrations of NH$_4^+$ were measured in triplicate using the phenol-hypochlorite method (Koroleff 1983). Duplicates of nitrate (NO$_3^-$), nitrite (NO$_2^-$), silicate (Si), and phosphate (PO$_4^{3-}$) were measured on a Lachat QuikChem 8500 autoanalyzer (Parsons et al. 1984). Urea was measured in duplicate using the manual monoxime method (Price and Harrison 1987). Dissolved primary amines (DPA), used as a proxy for amino acids (Keil and Kirchman 1991), were measured in triplicate on a Shimadzu RF-1501 spectrofluorometer following the o-phthaldialdehyde method (Parsons et al. 1984). Measurements of total dissolved nitrogen (TDN) and dissolved organic carbon (DOC) were made in triplicate by high-combustion on a Shimadzu TOC-V TNM (Sharp et al. 2002). Dissolved organic nitrogen (DON) was calculated by subtracting inorganic N
(NH$_4^+$, NO$_3^-$, and NO$_2^-$) from TDN (Bronk et al. 2000); the errors for all the terms were propagated to provide standard error for DON.

**Uptake rates**

Replicate subsamples of the melted cores were incubated with tracer (<10%) additions of $^{15}$N-labelled ammonium chloride (98.85% $^{15}$N) or potassium nitrate (98% $^{15}$N), along with $^{13}$C-labelled sodium bicarbonate (99% H$^{13}$CO$_3^-$; Cambridge Isotope Laboratories). Dual-labeled urea (98% $^{15}$N and 99% $^{13}$C) was added to the bottom ice layer during April of 2011, but not repeated in any other sections or season due to sea ice sampling limitations. The incubation bottles were placed in an environmental control chamber at ambient light (defined as the light levels measured in the water column immediately below the ice) and temperature for 24 hours (see Chapter 2 for complete details).

At the termination of the incubations, the samples were filtered through pre-combusted Whatman GF/F filters and frozen at -20°C until analyzed on a Europa Geo 20/20 isotope ratio mass spectrometer with an ANCA autosampler. Absolute uptake rates were calculated as per Dugdale and Goering (1967) and Hama et al. (1983) for N and C respectively. Specific uptake rates, which are simply the absolute rate divided by particulate N, remove any normalization to biomass. These rates describe the physiological ability of cells to assimilate N (Dugdale and Wilkerson 1986) and can give an indication of cells ability to assimilate N regardless of how many cells are present. The NH$_4^+$ and NO$_3^-$ pools were isolated at the end of the incubations by solid phase
extraction (Brzezinski 1987; Dudek et al. 1986) and the denitrifier method (Sigman et al. 2001) respectively. The atom percent enrichment of the isolated pools were used to correct the \( \text{NH}_4^+ \) and \( \text{NO}_3^- \) uptake rates for isotope dilution, calculate \( \text{NH}_4^+ \) and \( \text{NO}_3^- \) regeneration (Glibert et al. 1982), and nitrification rates (Ward 2008).

Results

Physical properties

During spring (April) sampling, the daily light cycle consisted of 20 hours of sunlight, clear skies, low wind, and air temperatures that hovered near -10°C. The water column was well mixed at a temperature of -1.8°C and salinity of 32; further details on water column conditions and N uptake are explored in Chapter 2. Ice depth was 130 cm with very little (<3 cm) snow covering the site. Temperatures in the ice core decreased linearly from the bottom of the core to the top, while brine salinity increased from a low of 41 located 5 cm from the ice-water interface to a high of 123 at the top of the ice (Figure 1). Based on the brine volume percent calculated from these parameters, there is a large amount of interstitial space at the bottom of the core (Golden et al. 1998), with permeability of the brine extending almost 80 cm up from the ice-water interface, excepting one point at 35 cm.

During January of 2012, the sun had remained below the horizon for 2 months prior to our sampling. Each day consisted of approximately 4 hours of twilight. In the days immediately preceding sampling, there were strong winds with daily temperatures ranging from -40 to -60°C. There was a brief respite on the day we obtained the ice cores,
with air temperatures reaching a low of -25°C. The water column remained well mixed during the winter, with water temperatures of -1.8°C and salinity of 34. The ice was only 60 cm, approximately half the depth of the spring sampling. Temperature in the core again reflected an essentially linear decline from the water to the air temperatures (Figure 1). The brine was much saltier than in April, ranging from 72 to 210. By 25 cm, the sea ice was impermeable to flow within the brine (Golden et al. 1998), which is interesting because our incubations were performed on sections to either side of that percolation barrier. There was visible sediment present in the bottom ice, but this was not quantified.

Community composition

In April, Chl a was low throughout the profile with the exception of the bottom ice layer, where there was a visible band of ice algae. The ice algae band measured 67.7 ± 2.9 and 415 ± 17.8 μg Chl a L⁻¹ in the bulk and brine-normalized ice, respectively (Figure 2). Mean Chl a in the remainder of the sampling depths in bulk sea ice was 0.11 μg Chl a L⁻¹, which was similar to Chl a found in the water column. The chlorophyll a-to-phaeophytin ratio (chl a:phaeo) can be used to give an indication of the physiological state of phytoplankton, with higher values indicating cells with fewer degradation products, and lower values indicating a state of degradation due to stress (Yentsch 1965). In the water column during April, the mean chl a:phaeo was 0.66, and bottom, middle, and upper sections of ice were 1.58, 0.95, and 0.64, respectively. Bacterial abundance generally mirrored Chl a, with a peak in the bottom ice, and a reduction further up the ice core. Bulk ice bacterial abundance was almost an order of magnitude lower than in the
water column, but higher by almost the same amount when calculating abundance as a function of the brine channel volume.

During January, there remained a Chl $a$ peak in the bottom ice, and whether in the bulk ice or brine-normalized, Chl $a$ in the sea ice was greater than the water column (Figure 2). While greatly reduced from April, there was still a community with Chl $a$ pigment present, although more degraded than in spring. The mean water column chl $a$ : phaeo was 0.33, and was 0.62, 0.37, and 0.15 in the bottom, middle, and upper sections of ice respectively. Bacterial abundance in the water column was not significantly different from spring. In the January sea ice, however, bacterial abundance was higher than spring and the water column at all depths. Once accounting for brine volume, the bacterial abundance in the sea ice is not significantly different between the three depth horizons.

Unlike the community present during April in the water column (Galand et al. 2009; Kirchman et al. 2010), Betaproteobacteria were well represented in the ice (Figure 3). In equal proportion were Alphaproteobacteria and Flavobacteria, with a smaller contribution of Gammaproteobacteria. A miniscule percentage (<1%) of probes were positive for crenarchaeota in the middle and upper ice, while not statistically significant from zero in the bottom ice. Winter CARD-FISH samples were not analyzed.

*Primary and bacterial production*

During April, absolute uptake of HCO$_3^-$ ($\mu$mol C L$^{-1}$ h$^{-1}$) in the bottom ice was more than 1000 times greater than in the water column, and close to two orders of
magnitude greater than in the upper two sections of ice (Table 2). Bacterial production was also highest in the bottom ice, but the bacterial to primary production ratio (BP:PP) was actually highest in the middle ice. Water column HCO$_3^-$ uptake was particularly low, while bacterial production in the water column was slightly higher than the middle and upper ice. This corresponds to an unusually high BP:PP of 7.0, although both BP and PP were very low in absolute terms.

In January we were unable to detect any primary production in the water column or the ice. Bacterial production was approximately an order of magnitude lower during winter than in spring, but still highest in the bottom ice. The middle and upper ice were not significantly different from each other, and again slightly lower than bacterial production measured in the water column.

**Nutrients – water column and bulk ice**

With the exception of urea and DPA, the water column had relatively high nutrient concentrations (see Chapter 2) compared to the bulk sea ice (Figure 4). In April, the organic components were at or below water column concentrations in the middle and upper ice but higher in the bottom ice. While NH$_4^+$, urea and overall DON all had maxima in the bottom ice, amino acids (i.e. DPA) and DOC are especially pronounced. It is interesting to note that the highest urea concentrations anywhere in the profile occur in the upper ice. The DOC:DON ratio is lower in the ice than the water column, but still above the Redfield ratio (Redfield 1934). The essential nutrients PO$_4^{3-}$ and Si were
almost zero in the ice. While \( \text{PO}_4^{3-} \) is low in the water column also, water column Si concentrations are over 40 \( \mu \text{M} \).

Winter concentration profiles of the ice and water column displayed a strikingly similar pattern to the spring (Figure 4), with a few caveats. In the bottom ice, \( \text{NO}_3^- \) concentrations were not as low as during the spring, and actually increased from the bottom to the top of the ice core. Urea concentrations did not increase to the same extent, and the DOC concentration was lower in the ice than in the water column. During winter, the DOC:DON ratio in the water column was almost double that of spring, and while lower overall in the ice compared to the water column, this ratio increased from the ice-water interface to the upper section of ice.

**Nutrients – brine normalized**

Sympagic organisms live in the brine channels of the sea ice, and therefore are not subject to the dilution effects of melted ice. Instead, they are exposed to much higher concentrations of ambient nutrients than the bulk ice described above and in Figure 4. When accounting for the brine channel volume, the relative pattern within the sea ice remains, but the absolute concentrations increased dramatically once the actual volume of the living space present in the ice is accounted for.

In April, \( \text{NH}_4^+ \) and \( \text{PO}_4^{3-} \) concentrations within the brine channels declined from the bottom to upper ice, while \( \text{NO}_3^- \) and Si concentrations increased in that upper section (Figure 5). In particular, \( \text{PO}_4^{3-} \) dropped from 4.8 \( \mu \text{mol P L}^{-1} \) to nearly zero (0.06 ± 0.08). At a mean of 8.3, the ratio of \( \text{NO}_3^-:\text{PO}_4^{3-} \) was below the Redfield ratio at every depth in
the water column and ice, with the exception of the middle ice. Organic constituents were especially high in the bottom ice layer. Concentrations of DOC reached $893 \pm 8.9 \mu\text{mol C L}^{-1}$, while DON peaked at $87.8 \pm 1.2 \mu\text{mol N L}^{-1}$. Both of these bulk organic pools declined further up the ice, keeping the DOC:DON ratio between 8.3 and 10.2. Urea and DPA combined comprised 26.7, 26.1 and 46.0% of DON in the bottom, middle, and upper sections respectively; DPA accounted for the majority of DON in the bottom ice, and urea in the other two sections.

As described above, the brine channels of upper ice were not connected to the lower sections of ice during winter (Figure 1). The nutrient profiles appear to reflect this lack of connection, with a “closed” system leading to higher nutrient concentrations in the upper ice (Figure 5). This is especially pronounced for $\text{NO}_3^-$, DOC, and Si. While this is also true for $\text{NH}_4^+$, DON, and $\text{PO}_4^{3-}$, the increases in upper ice concentrations were not nearly as substantial. Urea and DPA did not follow this pattern, and we did not observe the great increase from upper to bottom ice section seen during spring. Like spring, however, urea and DPA together accounted for a large percentage of the overall DON pool (51.9, 62.1 and 42.0% of total DON in the bottom, middle, and upper ice respectively), with urea providing the bulk of that. DOC:DON ratios were higher in January than in April at all three depths, and increased higher up in the core to a maximum of 15.0.
Nitrogen uptake and regeneration rates

During April, specific uptake of NH$_4^+$ and NO$_3^-$ were at maxima at the middle depth, but not significantly different between the bottom and upper ice (Figure 6). The middle ice section had a pronounced maximum of NO$_3^-$ specific uptake in the middle depth, with a value almost 4 times greater than the other two sections. Since biomass (Figure 2) was high in the bottom layer, absolute uptake rates of both NH$_4^+$ and NO$_3^-$ were highest there, dropping an order of magnitude in the two upper sections. These latter measurements are within range of water column absolute uptake of NH$_4^+$ (0.89 ± 0.34 nmol N L$^{-1}$ h$^{-1}$) and actually higher than water column NO$_3^-$ absolute uptake (0.28 ± 0.04 nmol N L$^{-1}$ h$^{-1}$). Urea uptake was only measured in the bottom ice during April of 2011 and had a specific uptake of 0.0027 ± 0.0007 d$^{-1}$ and absolute uptake of 1.98 ± 0.54 nmol N L$^{-1}$ h$^{-1}$. In the water column, specific uptake of urea (0.0018 ± 0.0003 d$^{-1}$) was not significantly different from the ice, and in both cases accounted for less than 5% of the measured specific uptake rates for all the substrates tested. Uptake of C from urea was below the detection limit.

As expected for the dark winter period, both specific and absolute uptake rates for NH$_4^+$ and NO$_3^-$ were considerably lower than in April (Figure 6). Specific uptake of NH$_4^+$ was not significantly different between depths in the January core. However, there is a trend of increasing NO$_3^-$ specific uptake with increased depth in the profile. These rates are near the limit of what can be reliably measured, and therefore have more substantial error than for the spring rates. In comparison to the water column, specific uptake of NH$_4^+$ at the 1 m depth is within range (0.00026 ± 0.00017 h$^{-1}$) of the ice, and
specific uptake of $\text{NO}_3^-$ is higher in the water ($0.00030 \pm 0.00017 \text{ h}^{-1}$). Similar to April, absolute uptake of $\text{NH}_4^+$ was highest in the bottom ice layer, while the two upper sections were not significantly different from each other and less than 25% of the bottom ice rate. For absolute uptake of $\text{NO}_3^-$ it was again lower in the bottom and middle ice, but slightly higher than absolute uptake of $\text{NH}_4^+$ in the upper ice.

Regeneration of $\text{NH}_4^+$ was highest in the bottom ice during spring. It was almost twice $\text{NH}_4^+$ absolute uptake in that same section, while also an order of magnitude higher than $\text{NH}_4^+$ regeneration in the other sections (Figure 6). Regeneration of $\text{NO}_3^-$ was also approximately double $\text{NO}_3^-$ absolute uptake in the bottom ice, but still only half the rate of $\text{NH}_4^+$ regeneration. With the exception of $\text{NH}_4^+$ regeneration in the upper ice, the bottom ice $\text{NH}_4^+$ regeneration and all of the $\text{NO}_3^-$ regeneration rates were higher than the water column ($\text{NH}_4^+$ regeneration = $27.8 \pm 1.18 \text{ nmol N L}^{-1} \text{ h}^{-1}$ and $\text{NO}_3^-$ regeneration was below detection limit). During January, regeneration of $\text{NH}_4^+$ was variable but not statistically different from zero in the bottom ice. In the water column, $\text{NH}_4^+$ regeneration rates were $13.8 \pm 4.76 \text{ nmol N L}^{-1} \text{ h}^{-1}$. All of the $\text{NO}_3^-$ regeneration rates during January were also highly variable, with a mean of $13.2 \text{ nmol N L}^{-1} \text{ h}^{-1}$ for all depths, which was only slightly lower than the mean for April ($14.9 \text{ nmol N L}^{-1} \text{ h}^{-1}$).

Nitrification, an important process during spring and winter in the water column of this area (Christman et al. 2011), was lower in the sea ice (Table 3) than the water column during both seasons. Rates were highest in the bottom ice during spring, and became progressively lower in the middle and upper ice. During January, nitrification was lowest in the bottom ice, and slightly higher in both the middle and upper ice.
Discussion

Most prior studies of sea ice biogeochemistry have focused on C cycling in bottom ice and investigated N cycling in the context of nutrient limitation. There is limited information on N uptake and regeneration in coastal regions (Cota et al. 1990; Cozzi 2008; Harrison et al. 1990; Kaartokallio 2001; Krell et al. 2003; Kuparinen et al. 2007; Priscu and Sullivan 1998; Rysgaard et al. 2008; Rysgaard and Glud 2004). In this study, we quantified the N cycling processes that underpin production within the ice at a western Arctic landfast site during spring and winter.

Biology

Ice algae biomass and production are concentrated on the underside and bottom few centimeters of sea ice (Horner and Schrader, 1982). During winter, flagellates make up the bulk of algal biomass in sea ice (Rózańska et al. 2009) and are succeeded by diatoms, which generally dominate the phytoplankton community in the entire core in both first year and multiyear ice during spring (Eddie et al. 2010; Werner et al. 2007). In addition to adaptations to brine channel conditions (Krembs et al. 2002; Krembs et al. 2000), ice algae can also modify the physical structure of the ice by the release of extracellular polysaccharide substances, which provides protection from freezing, osmotic stress, and potential refuge from predation (Krembs et al. 2011). It is not clear how phytoplankton survive the dark winter months, but possible overwintering strategies include survival by tapping into stored lipids and carbohydrates, a switch to heterotrophy, or formation of resting spores (Lizotte 2003). In our study, Chl a in the middle and upper
ice, although at low concentrations, were still higher than values in the water column, so it may be that the sea-ice environment itself acts as a refuge.

Previous studies indicate that bacterial and archaeal communities are evenly distributed throughout the ice column (Collins et al. 2010; Mock and Thomas 2005) and that respiration can exceed primary production (Deming 2010; Rysgaard et al. 2008) even during winter when bacterial growth is extremely slow (Wells and Deming 2006). In our study, there was little difference in bacterial abundance and community structure with depth of ice during spring, which could be explained by presence of brine channel connections between the different depths, inclusion into freezing ice by the same pelagic seed population, or competitive exclusion of other groups. Prior studies found more than 10% of Arctic surface water prokaryotic community to be Crenarchaeota during the winter, which decreases during spring and summer (Alonso-Sáez et al. 2008; Kirchman et al. 2007). Crenarchaeota are also negatively correlated to sediment loads (Galand et al. 2008). In the central Arctic, Alpha- and Gamma-proteobacteria and cytophaga account for more than 80% of clades in first year ice (Junge et al. 2004; Junge et al. 2002), with archaea only showing up in winter (Collins et al. 2010). Relative abundance of bacteria have been shown to increase as temperature decreases within the ice (Junge et al. 2004). Our results generally agree with prior studies, although Betaproteobacteria were numerically more important in our samples than Gammaproteobacteria. It is interesting to note that of the five groups we probed for (crenarcheota, gamma-, alpha-, beta-proteobacteria and bacteroides), almost 99% of DAPI cells are represented by eubacteria (probe EUB338; Table 1) in the bottom ice, but only 78% in the upper two sections.
indicating that there is a slightly different community in the top two layers that didn’t hybridize well with the probes we used.

Primary and secondary production

April Chl $a$ and absolute uptake of $\text{HCO}_3^-$ in the bottom ice was on the lower end of the spectrum of other bottom sea ice primary production measurements in our study region (Lee et al. 2008) and the Chukchi Sea generally (Gosselin et al. 1997; Gradinger 2009), highlighting the extreme seasonal productivity of the sea ice in the Arctic. Within the ice, there are relatively few measurements of primary production. In Barents Sea bottom ice, Mock and Gradinger (1999) measured primary production ranging from 0.25 - 7.11 mg C m$^{-2}$ d$^{-1}$. Similar studies in the Chukchi Sea found a range from 0.24 – 55.2 mg C m$^{-2}$ d$^{-1}$ (Gradinger 2009) and 0.5 – 310 mg C m$^{-2}$ d$^{-1}$ (Gosselin et al. 1997). It is likely that our lower rates (Table 2) were due either to the patchiness or that we sampled prior to those other studies and prior to the full development of the spring bloom in this region, which culminated in late May (A. Juhl, pers. comm.).

The prokaryotic community was active throughout the water and ice columns during both seasons, as indicated by a cross-seasonal range of bacterial production of 0.1 – 0.22 $\mu$g C m$^{-2}$ d$^{-1}$. In sea ice of the Amundsen Gulf (Canadian Arctic), Nguyen and Maranger (2011) found highly variable bacterial production, but with a mean (4.3 and 7.1 $\mu$g C L$^{-1}$ d$^{-1}$ during March and July respectively) higher than our study. Sea ice BP:PP in our study (0.03 – 0.13) was within range of ratios found in the summer (August) water column of the Chukchi Sea (0.04 - 0.10; Cota et al. 1996; Rich et al. 1997), but low
compared to spring water column BP:PP on the shelf of this region (0.05 - 0.96; Kirchman et al. 2009). In fact, the BP:PP in this study was an order of magnitude lower than the BP:PP found in winter Antarctic sea ice (Helmke and Weyland, 1995). These are the first measurements we know of for bacterial production in landfast sea ice of Alaska, and the lower numbers could simply reflect geographic or seasonal differences with prior studies.

Nutrients

The accepted paradigm of nutrition for sympagic organisms, at least in the bottom ice layer, is the physical exchange of nutrients with the water column (Thomas and Dieckman 2002; Thomas et al. 2010). However, inorganic nutrients and metals tend to be enriched in sea ice compared to the water column (Loose et al. 2011; Thomas et al. 1995; Tovar-Sánchez et al. 2010). The exception to this excess is NO$_3^-$, which is thought to have limited uptake due to low availability, high NH$_4^+$ concentrations inhibiting NO$_3^-$ uptake, and poor cellular transport at low temperatures (Cochlan and Bronk 2001; Pineault et al. 2013; Priscu et al. 1989; Priscu and Sullivan 1998; Smith et al. 1997). Limitation of biological production by low NO$_3^-$ concentrations, however, is equivocal, as it could be a function of geography, temperature, or light conditions (Harrison et al. 1990; Lee et al. 2010). In our study, we found no evidence of any nutrient limitation (as defined by concentrations of any macronutrient below 0.05 μM) in the ice, with the exception of possibly PO$_4^{3-}$ during spring, which fell to near zero in the middle and upper ice (Figure 5).
Dissolved organic N sources have received relatively less attention than inorganic N in sea ice, and should not be overlooked as an alternative or supplement to inorganic N (Conover et al. 1999; Harrison et al. 1990). High extracellular DNA concentrations occur in Arctic sea ice (Collins and Deming 2011) and recent work shows amino acids are important source of nutrition to the coastal Arctic microbial community, especially during winter (Chapter 2; Alonso-Sáez et al. 2008; Nikrad et al. 2012). In this study, high urea and DPA concentrations, along with lower DOC:DON ratios, indicate relatively N-rich organic matter in the sea ice compared to the water column (Figure 4). Within the ice, concentrations of urea and DPA were both evenly distributed throughout the ice in January, but DPA was highly enriched in the bottom ice, and urea in the upper ice, during spring (Figure 5). With only one spring measurement of urea uptake in the bottom ice, which itself was very low, further study of urea and amino acid uptake is warranted.

*Absolute and specific uptake of NH$_4^+$ and NO$_3^-$*

Working in Resolute Bay, Canada during the summers of 1985 and 1986, Harrison et al. (1990) found a wide range of absolute uptake of NH$_4^+$ (2.64-36.15 µmol N L$^{-1}$ h$^{-1}$), NO$_3^-$ (0.87-80.04 µmol N L$^{-1}$ h$^{-1}$) and urea (2.00-5.37 µmol N L$^{-1}$ h$^{-1}$) and concluded that the bottom ice community was not N limited. On the other side of the Arctic basin, in the landfast ice of the White Sea (Russia), Krell et al. (2003) found high DOC:DON, low N:P ratios, and limitation by NO$_3^-$, but conditions there are highly influenced by riverine inputs and therefore may be localized. In the coastal seas near
Barrow, Alaska, where this study took place, Lee et al. (2008) concluded that light, not inorganic N, was the primary environmental variable controlling ice algal C uptake.

Among N substrates tested in sea ice, one study from the Baltic Sea shows that bacterial communities have higher dependence on NH$_4^+$, as opposed to NO$_3^-$ (Kuparinen et al. 2011). In our study, absolute uptake of NH$_4^+$ was at least double absolute uptake of NO$_3^-$ in the bottom ice during both April and January, but reliance on NH$_4^+$ decreased in the middle and upper ice. There are limited comparable ice data, but this is counter to Reay et al. (1999) who used chemostat experiments with the water column community of the Southern Ocean and found increasing dependence on NH$_4^+$ with decreasing temperature. Despite our experimental conditions being very different, this discrepancy highlights the need for field studies within sea ice, where there are a number of variables (e.g. community composition, light, temperature) that all interact to influence the uptake rates.

In contrast to absolute uptake, specific uptake of NH$_4^+$ did not change much going up the ice profile, with the highest value in the middle ice during April and statistically equal rates in all ice depths during January (Figure 6). All of the specific uptake rates for NH$_4^+$ or NO$_3^-$ are outside of the range reported in the water and bottom ice of the Ross Sea during austral spring (Olson 1980). It is surprising that the lowest specific NO$_3^-$ uptake in both cases is in the bottom ice where primary production, which has traditionally been thought to preferentially derive from NO$_3^-$, is highest. These results indicate a high capacity for N uptake in the brine channels, even in the upper horizons. Because of dilution with ASW and the melting of bulk ice during the experiment, even
these rates should be considered conservative, as ambient nutrient concentrations were
diluted during the incubation set up. Because we corrected for isotope dilution, however,
our rates reflect the most accurate representation within the experimental conditions.
Isolation of NH$_4^+$ and NO$_3^-$ is labor intensive and costly. These analyses are necessary to
correct for isotope dilution, however, and necessary when calculating NH$_4^+$ uptake rates
(Glibert et al. 1982). There is increasing recognition that NO$_3^-$ uptake rates should be
corrected as well (Lipschultz 2008; Preston et al. 1998), especially in regions where the N
cycling processes are not well understood. Measurement of the isolated pools post-
incubation can also be used to directly calculate regeneration and nitrification rates (Ward
2008).

*Nitrogen regeneration*

In addition to uptake, nutrient concentrations in sea ice are generally considered
to result from exchanges with the water column and heterotrophic regeneration (Arrigo
and Thomas 2004; Gradinger and Ikävalko 1998; Thomas et al. 1995) and there has been
some circumstantial evidence for remineralization through indirect measurements
(Harrison et al. 1990; Riedel et al. 2007a; Riedel et al. 2007b). Here we were able to
directly measure an internal regenerated N pool that is not dependent on outside sources.
Heterotrophic bacteria constitute a large portion of sea ice biomass (Deming 2010) but
because of the isolated nature of many of the brine pockets in the ice, the microbial loop
is not able to fully develop, leading to accumulation of organic C (Lizotte 2003). If
active, however, heterotrophic activity would remineralize both organic C and N.
Kirchman 2000). Direct quantification of NH$_4^+$ and NO$_3^-$ regeneration in sea ice has heretofore not been tested polar sea-ice.

In this study, regeneration rates of NH$_4^+$ are generally higher than rates of NH$_4^+$ absolute uptake at each depth. The pattern of mean NH$_4^+$ regeneration closely aligns with NH$_4^+$ concentrations at every depth for both April and January. Curiously, NH$_4^+$ regeneration was below our detection limit in the middle ice in April and in the bottom ice during January. When not below the detection limit, NH$_4^+$ regeneration was 1.6 – 33 times greater than absolute uptake of NH$_4^+$. Measured by changes in NH$_4^+$ concentration over time, Riedel et al. (2007b) calculated an extremely high NH$_4^+$ regeneration rate of 200 nmol L$^{-1}$ h$^{-1}$ in new sea ice (<7 cm thick) during autumn, contributing 67% of the sea-ice NH$_4^+$ concentrations. In our study, such high NH$_4^+$ regeneration in April bottom ice might indicate very high turnover of NH$_4^+$, but without time course sampling during our 24 hour incubation period, we potentially underestimate NH$_4^+$ regeneration in other samples, as the NH$_4^+$ is recycled faster than our sampling regime could account for (Dickson and Wheeler 1995). Additionally, in temperate marine ecosystems when NO$_3^-$ is replete, diatoms release NH$_4^+$ with rapid increases in irradiance and decreases in ambient temperature (Lomas et al. 2000), which potentially helps to explain high NH$_4^+$ turnover. In our study, we were careful to prevent light, temperature, and osmotic shock, and did not subject the communities to either of these conditions, but it remains a possibility that sea ice microorganisms are releasing NH$_4^+$ due to some stress response. What is clear is the NH$_4^+$ regeneration rates, when above our detection limit, indicates a highly regenerative system, with tight coupling between autotrophic and heterotrophic
production. Whether \(NH_4^+\) regeneration in sea ice is highly variable or suffering from sparse sampling has yet to be determined.

During both April and January, \(NO_3^-\) regeneration was an order of magnitude higher than absolute uptake of \(NO_3^-\) at all three depths in the ice (Figure 6). In combination with the isolated brine pocket found in the January upper ice, elevated \(NO_3^-\) regeneration helps explain the high \(NO_3^-\) concentrations we saw in January upper ice (Figure 5), as it was 69 – 165 times the \(NO_3^-\) absolute uptake rate. Strangely though, nitrification rates only account for \(24 \pm 17\%\) of the \(NO_3^-\) regeneration rates that we measured. It is unclear what process other than nitrification would increase \(NO_3^-\) concentrations during the incubations. Cells could potentially lose \(NO_3^-\) in response to stressors, such as grazing, virally-induced lysis, or elevated light (Bronk and Steinberg 2008; Lomas et al. 2000; Wells and Deming 2006). While we controlled light in our experimental setup, grazers were not excluded and viruses were not enumerated. More research is needed to understand if high \(NH_4^+\) and \(NO_3^-\) regeneration rates are a universal feature of sea ice and what conditions might influence it.

In addition to regeneration, transformational processes between N compounds can have important implications for sympagic biology. Heterotrophic activity is clearly high during both spring and winter, and denitrification and annamox have been found in Arctic sea ice despite generally high oxygen availability (Rysgaard et al. 2008; Rysgaard and Glud 2004). On the other hand, nitrification could help explain high \(NO_3^-\) by oxidation of \(NH_4^+\) into the \(NO_3^-\) pool. Nitrifiers are present in large numbers in central Arctic waters (Bano and Hollibaugh 2000), but their rates in sea ice have only been directly
measured in Antarctic landfast ice, where it was found that NH$_4^+$ oxidizers could account for a significant portion of heterotrophic C fixation and provide sufficient NO$_3^-$ for uptake (Priscu et al. 1990). Nitrification rates in this study were highest in the bottom ice during spring, and were an order of magnitude lower in the upper section (Table 3), potentially indicating light limitation of the process (Ward et al. 1984). On the other hand, nitrification remained lower than the water column during the dark winter, which would point to some other environmental factor influencing this process. We have limited information on community composition in our ice samples, but Betaproteobacteria, of which prominent nitrifiers (Nitrosomonas) are a part, were a large percentage of our identified spring ice clades. In April upper ice, we also found low abundance of Crenarchaeae, which are globally important nitrifiers (e.g. Francis et al. 2005; Wuchter et al. 2006). During January, when temperature decreased rapidly towards the surface, nitrification rates in all three sections were not statistically different from each other, which confirm the results from water column manipulation experiments.

Conclusions

Sympagic primary productivity is expected to increase in a warming climate until the N supply from the water column becomes limiting (Arrigo et al., 2008, Carmack and McLaughlin 2011; Gradinger 2009, Pabi et al. 2008). But organismal adaptations may be acting to retain more nutrients in the ice (Krembs et al. 2011), and our study shows that N regeneration within the ice can match or exceed absolute uptake rates. As ice extent and volume continues to be reduced (e.g. Maslowski et al. 2012; Stroeve et al. 2007; Wang
and Overland 2009), timing changes in the onset of the spring bloom will have impacts on food webs throughout the Arctic (Kahru et al. 2011; Tremblay et al. 2006), and one would expect remineralization in spring ice and the water column to increase as more organic material is available to the heterotrophic bacterial community, further reducing the capability of the Arctic Ocean to act as a CO₂ sink in the future (Cai et al. 2010; Else et al. 2013; Vaquer-Sunyer et al. 2010).

In this study, we were able to resolve sea ice N uptake and regeneration processes in landfast ice of the Chukchi Sea during winter and spring. The high concentrations of nutrients and diversity of organisms within the brine channels indicate that it is a thriving ecosystem, even within the constraints of variable light regimes, low temperatures and high salinities. While ice algae are widely considered to be an important driver of marine Arctic food webs, this study shows that the microbial N transformations throughout the ice help support their growth, and that biogeochemical cycling above the layer of ice algae is at least as important as in the water column and needs to be taken into account when attempting to understand nutrient dynamics in the Arctic, now and in the future.

Acknowledgments
This project was supported by NSF grant # ARC-0909839. Many thanks to P. L. Yager for use of her ice auger, Q. R. Roberts, K. Sines, and C. Williams for their tireless efforts in the laboratory, and R. E. Sipler for enduring the weather conditions out on the sea ice, along with many discussions that have improved the manuscript. The field component of

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References


Grebmeier JM, Cooper LW, Feder HM, Sirenko BI (2006) Ecosystem dynamics of the Pacific-influenced Northern Bering and Chukchi Seas in the Amerasian Arctic. Prog Oceanogr 71 (2-4):331-361.


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### Tables

**Table 1. Sequences of oligonucleotides (16S rRNA) used as FISH probes.**

<table>
<thead>
<tr>
<th>Probe name</th>
<th>Target group</th>
<th>Sequence (5'-3')</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>EUB338</td>
<td>Bacteria</td>
<td>GCTGCCTCCCCTTAGGAGT</td>
<td>Amann et al. (1990)</td>
</tr>
<tr>
<td>CREN554</td>
<td>Crenarchaeota</td>
<td>TTAGGCTCAATAATCMTCCT</td>
<td>Massana et al. (1997)</td>
</tr>
<tr>
<td>GAM42a</td>
<td>Gammaproteobacteria</td>
<td>GCCTGCACACATCGGT</td>
<td>Manz et al. (1992)</td>
</tr>
<tr>
<td>ALF968</td>
<td>Alphaproteobacteria</td>
<td>GGTAAGGTTCCTCGCGTT</td>
<td>Glöckner et al. (1999)</td>
</tr>
<tr>
<td>BET42a</td>
<td>Betaproteobacteria</td>
<td>GCCTCCCACTTCGTT</td>
<td>Manz et al. (1992)</td>
</tr>
<tr>
<td>CF319a</td>
<td>Bacteriodes</td>
<td>TGGTCCGTGTCTCAGTAC</td>
<td>Weller et al. (2000)</td>
</tr>
<tr>
<td>NON338</td>
<td>Control</td>
<td>ACTCCTACGGGAGGCAGC</td>
<td>Wallner et al. (1993)</td>
</tr>
</tbody>
</table>

1 complimentary to EUB338.
Table 2. **Specific and absolute C uptake rates.** Rates and standard deviations for HCO$_3^-$ and $^3$H-leu uptake. Chl $\alpha$ and bacterial abundance for the sea ice depths are brine-corrected concentrations. BDL is below detection limit. BP:PP values could not be calculated for January 2012 since no HCO$_3^-$ uptake was detected in the winter incubations. Negative values denote water column depths.

<table>
<thead>
<tr>
<th>Month</th>
<th>Depth (cm)</th>
<th>Chl $\alpha$ (µg L$^{-1}$)</th>
<th>Bacterial abundance (10$^8$ cells L$^{-1}$)</th>
<th>Particulate C (µmol C L$^{-1}$)</th>
<th>HCO$_3^-$ specific uptake (h$^{-1}$) x 1000</th>
<th>HCO$_3^-$ absolute uptake (nmol C L$^{-1}$ h$^{-1}$)</th>
<th>$^3$H-leu uptake (pmol leu L$^{-1}$ h$^{-1}$)</th>
<th>BP:PP</th>
</tr>
</thead>
<tbody>
<tr>
<td>April</td>
<td>35</td>
<td>6.0 ± 2.1</td>
<td>62 ± 0.2</td>
<td>4.81 ± 0.31</td>
<td>1.3 ± 0.2</td>
<td>6.32 ± 1.11</td>
<td>0.18 ± 0.03</td>
<td>2.38 ± 0.19</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>3.3 ± 0.4</td>
<td>72 ± 2.2</td>
<td>4.51 ± 0.24</td>
<td>1.4 ± 0.3</td>
<td>6.19 ± 1.14</td>
<td>0.12 ± 0.07</td>
<td>6.19 ± 0.34</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>415 ± 17</td>
<td>148 ± 2.1</td>
<td>23.4 ± 1.72</td>
<td>6.5 ± 0.8</td>
<td>153 ± 17.9</td>
<td>4.41 ± 0.51</td>
<td>37.9 ± 2.16</td>
</tr>
<tr>
<td></td>
<td>-400</td>
<td>0.1 ± 0.0</td>
<td>1.8 ± 0.0</td>
<td>2.07 ± 0.18</td>
<td>0.49 ± 0.13</td>
<td>0.15 ± 0.01</td>
<td>0.004 ± 0.002</td>
<td>8.23 ± 0.14</td>
</tr>
<tr>
<td>January</td>
<td>35</td>
<td>0.7 ± 0.0</td>
<td>149 ± 0.6</td>
<td>15.5 ± 3.09</td>
<td>BDL</td>
<td>BDL</td>
<td>BDL</td>
<td>0.99 ± 0.24</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0.6 ± 0.0</td>
<td>227 ± 1.7</td>
<td>10.4 ± 0.45</td>
<td>BDL</td>
<td>BDL</td>
<td>BDL</td>
<td>1.18 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>16 ± 0.0</td>
<td>165 ± 4.0</td>
<td>10.2 ± 2.18</td>
<td>BDL</td>
<td>BDL</td>
<td>BDL</td>
<td>6.07 ± 0.20</td>
</tr>
<tr>
<td></td>
<td>-100</td>
<td>0.0 ± 0.0</td>
<td>1.7 ± 0.0</td>
<td>23.4 ± 1.72</td>
<td>BDL</td>
<td>BDL</td>
<td>BDL</td>
<td>1.75 ± 0.32</td>
</tr>
</tbody>
</table>
Table 3. Nitrification rates. Measured nitrification rates and standard deviations in the sea ice and water column (denoted by negative values) for spring and winter sampling.

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>Nitrification rate (nmol N L(^{-1}) h(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>April</td>
</tr>
<tr>
<td>35</td>
<td>0.60 ± 0.06</td>
</tr>
<tr>
<td>20</td>
<td>2.21 ± 2.09</td>
</tr>
<tr>
<td>5</td>
<td>5.40 ± 0.28</td>
</tr>
<tr>
<td>-100</td>
<td>5.40 ± 0.28</td>
</tr>
<tr>
<td>-400</td>
<td>24.9 ± 16.6</td>
</tr>
</tbody>
</table>
Figures

Figure 1. Physical conditions of the sea ice and brine channels. Temperature (open triangles), brine salinity (open squares), and brine channel volume percent (filled circles) profiles for cores during spring (left panel) and winter (right panel). The profiles are for the full depth of the ice cores, which was 130 cm in spring, but 60 cm in winter. The vertical dashed lines are the 5% brine channel volume percent, which corresponds to the lower limit of permeability in the ice core (Golden et al. 1998). Note the different scales for salinity and temperature. A depth of zero corresponds to the ice-water interface.
Figure 2. Chlorophyll a and bacterial abundance in sea-ice and water column. Chl a and bacterial abundance for spring (a) and winter (b). Error bars indicate standard deviation; when no error bars are seen, they were smaller than the symbol. The dashed horizontal line indicates the ice-water interface; the sea ice values are above and water column values are below that line. The inset boxes are brine-normalized values for the Chl a and bacterial abundance of just the sea ice communities; note the different scales from the larger plots, and between the seasons.
Figure 3. Community composition of sea ice communities. Means of CARD-FISH results as a function of overall percentage of the sea ice community for spring. Probes used for alpha- (ALF), beta- (BET), and gamma- (GAM) proteobacteria, bacteriodes (CF), and crenarchaeota (CREN). Positive (eubacteria) control discussed in Results and negative (antisense) control not shown.
Figure 4. Bulk nutrients in the ice and water column. Ambient nutrient concentrations in the water column and bulk ice for spring (upper panel) and winter (lower panel). The dashed line indicates the ice-water interface; the sea ice values are above and water column values are below that line. Symbols, as noted in the bottom panel, indicate NH$_4^+$ (filled circles) and NO$_3^-$ (open circles), urea (filled triangles) and DPA (open triangles), DOC (filled squares) and DON (open squares), PO$_4^{3-}$ (filled diamonds) and Si (open diamonds). Error bars indicate standard deviation; some error bars are smaller than the symbol.
Figure 5. Brine normalized nutrient concentrations in the sea ice. Concentrations of ambient nutrients in the sea ice brine channels for spring (upper panel) and winter (lower panel). Symbols, as noted in the bottom panel, indicate NH$_4^+$ (filled circles) and NO$_3^-$ (open circles), urea (filled triangles) and DPA (open triangles), DOC (filled squares) and DON (open squares), PO$_4^{3-}$ (filled diamonds) and Si (open diamonds). Error bars indicate standard deviation; some error bars are smaller than the symbol. The DOC:DON axis does not include a break.
Figure 6. Uptake and regeneration rates of $\text{NH}_4^+$ and $\text{NO}_3^-$. For each sampling depth (centered on 5, 20, and 35 cm from the ice-water interface), values of specific uptake ($V$), absolute uptake ($\rho$) and regeneration ($R$) of $\text{NH}_4^+$ (black) and $\text{NO}_3^-$ (gray) are given for spring (top row) and winter (bottom row). BDL is below the detection limit. Note different x-axis scales for different seasons (upper and lower panels), especially for $V$, which is an order of magnitude lower during January.
Chapter 6 – Conclusion
This chapter summarizes the principal findings and theorizes about potential impacts on our understanding of the coastal Chukchi Sea ecosystem as well as the potential for future biogeochemical changes in the ecosystem. The research was full of both scientific and physical adventures, which helped to add to our knowledge of nitrogen (N) cycling, but also sparked new ideas for how to extend the scope of the research questions. In that regard, limitations, lessons learned and future directions of the research are also presented.

The essential issue that was addressed was the limited data on N uptake and regeneration across the Arctic, despite the hypothesized importance of these processes in sea ice and during winter. Furthermore, there is very little data from anywhere in the Arctic on N uptake and regeneration rates within the sea ice matrix (i.e. not just the bottom ice) and in the water column beneath sea ice (i.e. winter and spring). This lack of knowledge is especially striking in the shallow regions away from large rivers such as the Chukchi Sea. Even though it is known that the Arctic is warming faster than anywhere else on Earth (e.g. Serreze and Francis 2006), there is very little empirical information on how increased temperatures will impact N cycling.

Chapter 2 addresses the shortage of data sets on N uptake and regeneration in the western coastal Arctic. The sampling regime consisted of experiments to quantify N uptake rates in the water column and sea ice during January, April, and August. Reported herein is evidence of the importance of both NH$_4^+$ and amino acids compared NO$_3^-$ in this system. Chapter 3 presents data that show this preference holds for both phytoplankton fractions and the bacterial LNA and HNA populations. Chapter 5 extends this
generalization further to the landfast sea ice community. This preference for NH$_4^+$ is also different from N uptake measured in other Arctic regions, where NO$_3^-$ uptake tends to be more important. While it appears that NH$_4^+$ is preferentially used in the Chukchi Sea generally, it may be a feature of coastal regions across the Arctic.

Polar amplification of climate change is rapidly changing the system. Temperature increases could potentially cause changes in biogeochemical rates and is already triggering declines in multiyear sea ice. The results from Chapter 4 illustrate the magnitude of changes to NH$_4^+$ uptake and nitrification rates that may be expected to happen along with changes in temperature. Rates of NH$_4^+$ uptake increased with temperature and were positively correlated to ambient NH$_4^+$ concentrations, but were likely to be inhibited by light. If sea ice recedes earlier in the year, it is likely to precipitously reduce nitrification rates and therefore NO$_3^-$ concentrations. This could potentially reduce the timing and magnitude of the spring phytoplankton bloom, which would have drastic impacts on the Arctic food web.

The results from Chapter 5 showed that N uptake rates within the sea ice are typically greater than the water column during January and April. The landfast first year ice could be a harbinger of sea ice biogeochemistry in the future. Although there is little comparable data from multiyear ice, the light regime within the thinner sea ice is likely to promote changes in the timing and magnitude of the spring phytoplankton bloom, thereby increasing biomass and N uptake rates throughout the ice. With greater biomass, the microbial community would be expected to respond with greater N regeneration rates and
a larger fresh organic matter pulse to the water column, further enhancing primary production throughout the Arctic (Arrigo et al. 2008).

Research limitations

Our research plan focused on a wide variety of N substrates and sampling across different seasons and was heavily influenced by the physical conditions present in Barrow, including the location of impassable pressure ridges, stability of the sea ice, and avoidance of the annual bowhead whale hunt of the local community. During the summer, high seas and low visibility made it difficult at times to procure water for sampling. Some of these difficulties were anticipated in the sampling plan and strategies were devised to overcome any obstacles, within the confines of physical safety for all involved.

It could be argued that to truly understand seasonal dynamics and the intense pulses of activity known to occur in the region extended continuous time sampling the region would be warranted. This was done for the CASES project in the southeastern Beaufort Sea, which overwintered aboard the CCGS *Amundsen* (details first presented in Tremblay et al. 2008). Our study was funded to visit at discrete time periods and we performed as much sampling as we could within the limitations of what was funded. While we attempted to be at our sampling site as close to the time of the historical phytoplankton bloom period, we were early by a few weeks each year (A. Juhl, pers. comm.)
As described in Chapters 2 and 3, we used size fractionation to discriminate uptake by phytoplankton from that by bacteria. The biomass in our sample region is low, necessitating large volumes of water to filter in order to get enough mass for a reliable reading on our mass spectrometer. We attempted to capture bacterial cells passing through the GF/F filters by terminating the incubations over 0.2 µm silver filters but found the filtration times were greater than 1 hour each, and would have compromised our ability to perform multiple experiments with a variety of N forms. Additionally, such long filtering times risk exposing the microorganisms to artificial conditions (i.e. light, temperature) of the laboratory and making the end point of the incubations unclear, factors that we had worked hard to eliminate.

We attempted to mitigate size fractionation issues with parallel incubations intended for flow cytometric sorting, using 0.2 µm Supor filters. We hoped to identify phytoplankton and bacterial groups to measure their respective uptake rates unambiguously. Unfortunately, cell fluorescence was so low such that there was no clear separation of the phytoplankton and bacterial populations, unlike what we have observed in the other estuarine, coastal and ocean systems we have sampled. It is currently unclear whether the lack of separation has to do with the region we sampled or because of a problem with our sampling methodology. Now that the Bronk lab has a high-speed sorter in house, we intend to perform method tests to perfect our sample preparation processes. In an effort to make lemonade out of lemons, the sort procedure was adjusted to interrogate low nucleic acid and high nucleic acid populations, as described in Chapter 3. While not the original goal of the sampling procedure, the results of our LNA and HNA
sorting should result in compelling evidence of the ecological significance of the bacterial subpopulations.

**Future directions**

The research presented in this dissertation has provided information on N cycling processes in the coastal western Arctic. In addition to adding to the body of knowledge on polar biogeochemistry, it has raised some fundamental questions about how the coastal Chukchi Sea ecosystem functions at the microbial scale. Given the opportunity, future experiments should expand on the knowledge gained by connecting the discrete sampling of this study with connections to the wider ecosystem temporally and spatially along with connections to other elemental cycles and food web dynamics. As mentioned above, greater continuous coverage of N uptake, especially during the spring phytoplankton and bloom periods, could provide a better mechanistic understanding of the seasonal dynamics of the Chukchi Sea. There is great potential to recruit the local community, in collaboration with a qualified lab, to perform basic monitoring of nutrients and physical conditions. This kind of long-term and detailed monitoring would be invaluable for efforts to evaluate changes and provide context for more targeted uptake studies. In addition to more temporal coverage, wider spatial sampling could provide better understanding of variability in the region. Our sampling site was within a few miles of the flaw lead. How do ice conditions and uptake change even in that short distance? Barrow is located just west of the convergence of the Chukchi and Beaufort
Seas, which have vastly different conditions. Future work could focus on the gradients between these two seas along with onshore to offshore gradients.

Midway through this project, Christman et al. (2011) published the first report of winter nitrification rates in polar waters. Chapter 3 corroborated the vast seasonal difference in rates and furthermore explicitly tested the impact of warming temperatures on $\text{NH}_4^+$ uptake and nitrification. Additional work is warranted on warming impacts of $\text{NO}_3^-$, urea, and amino acid uptake. Climate change impacts will also influence light, pH and stratification. Incorporating these elements into future studies of N uptake would provide some predictive power to climate change modeling in the Arctic and beyond.

The N uptake in this study focused on the water column and sea ice. Sampling took place in a shallow system, but we did not account for any benthic processes. Future work should attempt to directly link biogeochemical cycling in all three (presumably) closely linked systems: the benthos, water column, and sea ice. Additionally, riverine inputs to the Arctic have great influence on the freshwater budget and nutrient regimes of the basin (Aagaard and Carmack 1989; Holmes et al. 2012). Barrow, however, is located far from these influences. While it is likely that the Yukon and Mackenzie Rivers have some impact on this region, it is currently unclear what role overland or submarine flow have on nutrient inputs there.

Flow cytometric sorting provides an opportunity to connect specific populations of microorganisms with their uptake rates (Lomas et al. 2011), which is otherwise methodologically difficult. An alternate avenue would be to incorporate genomic studies with uptake. While not part of this dissertation, samples were taken for stable isotope
probing to clearly indicate which microorganisms actively incorporate the \( ^{15}\text{N} \) label. Molecular data could provide more detail on the organisms present and their physiological capabilities. Combining genomic work with uptake studies in the Arctic has already provided new information on the link between urea and nitrification (Alonso-Sáez et al. 2012) and \( \text{N}_2 \) fixation (Blais et al. 2012). Nitrification measurements have been reported extensively herein, but there is limited information on \( \text{N}_2 \) fixation. The Bronk lab recently made \( \text{N}_2 \) fixation measurements and found that it is low but present in the summer and that this may be a relatively new phenomenon (Bronk et al. in prep.). Could this be an indication that the system has become more \( \text{N} \)-limited during the latter half of the twentieth century? To what extent, under what conditions, and what organisms are responsible for \( \text{N}_2 \) fixation in the Arctic?

As presented in Chapter 5, there was a discrepancy between measured \( \text{NO}_3^- \) regeneration and nitrification rates. There are few \( \text{NO}_3^- \) regeneration rates reported throughout the literature, making a comparison difficult. Additionally, many reported rates of uptake for \( \text{NH}_4^+ \) and \( \text{NO}_3^- \) are not corrected for isotope dilution, and instead account for the former with the equations of Kanda et al. (1987) and the latter not at all. The limited information on isotope dilution correction across a variety of oceanographic areas cries out for an analysis of the difference between measured and calculated dilution effects.

**Synopsis**

The physical changes happening in the Arctic are having a profound effect on biogeochemical cycling. With this dissertation research, empirical data has been
presented on N uptake rates and regeneration in the water column and sea ice of the western coastal Arctic. It has added to the field of Arctic biogeochemistry, expanding understanding of N cycling processes in the western coastal Arctic and provided a baseline for future studies. The research has undertaken the task of describing N cycling in the water column, including size fractionation of phytoplankton, detailed N uptake rates of bacterial subpopulations, and potential future changes to these processes with respect to irradiance and nutrient concentration. The research has also expanded our knowledge of N cycling in landfast sea ice and provided the first direct measurements of NH₄⁺ and NO₃⁻ regeneration in Arctic sea ice. While the work presented here can stand on it’s own, there are many future avenues of research for comparison and further expansion of our knowledge of the rapidly changing Arctic ecosystem.

References


Vita

Steven E. Baer

Born in Boston, Massachusetts on April 18, 1973. Graduated from Framingham North High School in 1991. Received a bachelor’s degree in Social Thought and Political Economy from the University of Massachusetts, Amherst. Worked for a variety of technology companies with responsibilities including personnel management, business relations, marketing, and project management. Returned to undergraduate work in biology at the University of Massachusetts, Boston. Worked as an undergraduate assistant in the zooplankton ecology lab of Juanita Urban-Rich. Entered graduate school in the Physical Sciences department at the Virginia Institute of Marine Science, working in the nitrogen biogeochemistry lab of Deborah Bronk. Taught an undergraduate seminar course on Arctic Oceanography at the College of William and Mary. Defended doctoral dissertation in July 2013.