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LETTER

Preliminary estimates of the contribution of Arctic nitrogen fixation to the global nitrogen budget

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Scientific Significance Statement

Nitrogen fixation is an important source of "new" nitrogen to both freshwater and marine ecosystems where it supports biological production and balances aquatic nitrogen budgets. Although freshwater nitrogen fixation is relatively common at high latitudes, water column marine nitrogen fixation has historically been considered to be a mostly warm water, oligotrophic process. Here, we provide evidence to show that marine nitrogen fixation could be an important source of nitrogen to the seasonally nitrogen-limited Arctic Ocean, which could have far reaching implications for primary productivity and biogeochemical budgets in this rapidly changing ecosystem.

Abstract

Dinitrogen (N_2) fixation is the source of all biologically available nitrogen on earth, and its presence or absence impacts net primary production and global biogeochemical cycles. Here, we report rates of 3.5–17.2 nmol N L^{-1} d^{-1} in the ice-free coastal Alaskan Arctic to show that N_2 fixation in the Arctic Ocean may be an important source of nitrogen to a seasonally nitrogen-limited system. If widespread in surface waters over ice-free shelves throughout the Arctic, N_2 fixation could contribute up to 3.5 Tg N yr^{-1} to the Arctic nitrogen budget. At these rates, N_2 fixation occurring in ice-free summer waters would offset up to 27.1% of the Arctic denitrification deficit and contribute an additional 2.7% to N_2 fixation globally, making it an important consideration in the current debate of whether nitrogen in the global ocean is in steady state. Additional investigations of high-latitude marine diazotrophic physiology are required to refine these N_2 fixation estimates.

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Author Contribution Statement: DAB and RES designed the experiment. RES and MPS performed the experiments. RES, DAB, MPS, SEB, QNR, and MRM analyzed the data. DG determined the area and duration of ice-free regions. RES and DG calculated the potential contributions of N_2 fixation to local and global nitrogen budgets. RES and DAB wrote the paper with input from all authors.

Data Availability Statement: Data are available in the Biological & Chemical Oceanography Data Management Office (BCO-DMO) repository at http://www.bco-dmo.org/dataset/701789/data.

Net primary productivity in the Arctic Ocean is projected to increase in response to higher temperatures and consequent ice melt (Arrigo et al. 2012). Any increase in productivity, however, will require sufficient nitrogen to support it (Tremblay and Gagnon 2009). While several studies have speculated on changes to the Arctic nitrogen cycle and resultant effects on the primary producers that form the base of the food chain (Tremblay et al. 2008; Popova et al. 2012), the role of dinitrogen (N_2) fixation in the Arctic Ocean has received comparatively little attention.

Historically, marine N_2 fixation has been considered a warm-water process that occurs mainly in subtropical oligotrophic gyres and tropical seas, where it provides a new source of nitrogen to the system (Sohm et al. 2011). Recent work has shown, however, that N_2 fixers (Moisander et al.

Table 1. Site location and physical characteristics.

Station	Longitude (N)	Latitude (W)	Water column depth (m)	Sample collection depth (m)	Time started (local)	Temperature (°C)	Salinity
Marine							
1	71°20′40″	156°41′25″	17.1	4	19:10	5.0	30.5
2	71°20′40″	156°41′25″	17.5	8	15:16	4.7	30.4
3	71°20′36″	156°39′30″	8.0	2	20:38	4.6	30.4
4	71°20′40″	156°41′25″	17.5	8	23:57	4.5	30.4
8	71°19′02″	156°50′18″	ND	8	10:30	ND	30.4
Estuarine							
5	71°7′8″	156°54′24″	ND	Surface	15:53	6.7	12.0
6	71°7′45″	157°1′10″	ND	Surface	16:04	6.2	15.0
7	71°8′45″	157°3′45″	ND	Surface	16:18	6.0	20.1

Marine stations were located in the coastal Chukchi Sea (2.5 km northwest of Barrow, Alaska) and estuarine stations were in Walakpa Bay (20 km southwest of Barrow, Alaska). The local time of day when incubations was started is presented based on as 24-h clock.

2010; Blais et al. 2012; Díez et al. 2012; Fernández-Méndez et al. 2016) and N_2 fixation (Blais et al. 2012; Mulholland et al. 2012) can occur at lower temperatures and higher latitudes than once thought and in areas where nitrogen is not limiting (Hamersley et al. 2011; Sohm et al. 2011) shifting the paradigm of where marine N_2 fixation occurs.

The *nifH* gene encodes for the nitrogenase enzymes, which is responsible for N_2 fixation processes. Targeted *nifH* gene and broad metagenomic assessments have revealed the genetic capability for N_2 fixation in a variety of polar microbial communities. For example, *nifH* or species known to possess it have been detected in sea ice (e.g., Bowman et al. 2014; Fernández-Méndez et al. 2016), polar lakes (e.g., Toetz 1961; Shtarkman et al. 2013), the Arctic Ocean (e.g., Blais et al. 2012; Díez et al. 2012; Fernández-Méndez et al. 2016), glaciers (e.g., Telling et al. 2011), and polar soils (e.g., Solheim et al. 1996; Dickson 2000). These investigations provide compelling evidence that diverse polar microbial communities have the genes required to fix nitrogen. Fewer studies provide actual rates of marine N_2 fixation that can be used to estimate the amount of nitrogen contributed to global nitrogen budgets (e.g., Knowles and Wishart 1977; Haines et al. 1981; Gihring et al. 2010; Blais et al. 2012).

The goal of this study was to determine the rate of N_2 fixation in the surface mixed layer of the coastal Alaskan Arctic and to estimate how much nitrogen this process could contribute to local and global nitrogen budgets. These objectives were achieved using ^{15}N tracer techniques to measure rates of N_2 fixation during the summer under ice-free conditions at marine and estuarine sites in the Chukchi Sea, near Barrow, Alaska. The data presented here, and reported in Blais et al. (2012), were extrapolated to provide rough estimates of the potential range of fixed nitrogen contributed by Arctic marine N_2 fixation.

Methods

N_2 fixation

Field samples were collected under ice-free conditions 15–20 August 2011, in the coastal waters of the Chukchi Sea (salinity of 30) and Walakpa Bay (salinity of 12–20) near Barrow, Alaska. Rates of N_2 fixation were measured using the ^{15}N bubble addition method (Montoya et al. 1996). Gas-tight 1L glass KIMAX^{TM} media bottles (model # 611001000) capped with Wheaton^{TM} black open-top caps with gray butyl septa (model # 240680) were used for all incubations. The glass media bottles were acid washed (10% HCl), rinsed four times with high purity water (18.2 MΩ cm^{-1}) and combusted at 500°C for 4 h. The caps were submersed in a saltwater brine (approximately salinity of 60) for ~ 30 d, to condition them, and then acid washed (10% HCl) and rinsed with copious amounts of high purity water.

Seawater was collected using a low-pressure submersible electric pump (Johnson Pump model #16004) powered by a portable generator. Triplicate bottles were filled with seawater, capped and the ambient air bubbles were removed. The bottles were then amended with 1.2 mL (at a ratio of 1 mL of gas per 1L of seawater) of enriched (> 99%) $^{15}N_2$ gas purchased from Cambridge Isotope Laboratories (lot #11-10077). Samples were incubated for 24 h in environmental chambers located at the Barrow Arctic Research Center. To mimic in situ conditions, the temperature was set at 4.5°C and light levels to 50 μmol m^{-2} s^{-1} of light on a 24 h light cycle. No control incubation was done without added tracer gas. Sample locations, collection depths, and environmental conditions are presented in Table 1.

After 24 h, the incubations were terminated by filtering through 3.0 μm silver filters or pre-combusted (450°C for 2 h) GF/F filters with a nominal pore size of 0.7 μm. Filters were placed in sterile microcentrifuge tubes and stored

frozen at −20°C. Prior to analysis, filters were thawed and dried at 40°C overnight. Based on cell counts by flow cytometry, approximately 61% of the bacterial cells were retained by the GF/F (described in more detail in Baer et al. 2017). Isotopic measurements for ^{15}N fixation rates were analyzed on a Europa GEO 20/20 mass spectrometer with an ANCA-SL autosampler. The atom % enrichments for all N_2 fixation samples ranged from 0.3691 to 0.4463 and sample mass ranged from 9.25 μg N to 37.25 μg N. N_2 fixation rates were calculated using a mixing model (Montoya et al. 1996) and are reported as the mean ± the standard error for each site ($n = 3$) or grouped as marine ($n = 15$, five sites) or estuarine ($n = 9$, three sites) systems. The atom % enrichment of the particulate samples was measured against an atmospheric standard. They were not corrected for any variations in the natural abundance of the particulate material at the start of the incubation. This is a potential issue because particulate nitrogen has been shown to be enriched in ^{15}N in some regions of the coastal Arctic; in the summer of 2012, $\delta^{15}N$ values of 6.66‰ and 7.78‰ were reported at coastal sites in the Chukchi to the west of the sample sites from this study (Yu et al. 2014). These $\delta^{15}N$ values would not negate our observations.

We note that the bubble method has been shown to underestimate N_2 fixation based on gas solubility and may have biases against smaller symbiotic diazotrophs (Mohr et al. 2010; Großkopf et al. 2012). However, the overall magnitude of this underestimation is dependent upon the duration of the incubation and the temperature of the water in which the bubble is dissolved (Mohr et al. 2010). Culture experiments show that 75% of $^{15}N_2$ gas had reached equilibrium after 24 h at considerably higher temperatures (28°C) using the bubble method, and result in only a 15% underestimation in N_2 fixation rates (Mohr et al. 2010). Unlike the bubble method, the direct injection method (Mohr et al. 2010) requires a 10% dilution of the microbial community with filtered $^{15}N_2$ supersaturated seawater. Hypothesizing that fixation rates and diazotrophic abundance in the Arctic samples would be low, the bubble method was used to ensure that we did not dilute the community and therefore the rates of N_2 fixation. There have been no studies that report the bubble method overestimating N_2 fixation, therefore, the values reported here should be considered conservative estimates of N_2 fixation rates for the coastal Chukchi Sea.

We also note that contaminants ($^{15}NH_4^+$, $^{15}NO_3^-/NO_2^-$) have been measured in some $^{15}N_2$ gas stocks (Dabundo et al. 2014). If contaminated $^{15}N_2$ is used, measured N_2 fixation rates may be inflated due to the uptake of the contaminant and not fixation of the added $^{15}N_2$ gas. Dabundo et al. (2014) compared the level of ^{15}N gas contamination from three commonly used manufacturers of $^{15}N_2$. The $^{15}N_2$ gas used in our study was purchased from Cambridge Isotope Laboratories, which had the lowest level of contamination of all sources tested. The highest levels of contamination reported for Cambridge Isotope Laboratories, $^{15}N_2$ gas were not substantial enough to negate the rates we observed in the Arctic and would have accounted for a negligible proportion (≤ 0.3%) of the rates observed.

Nutrients

Ammonium concentrations were analyzed using the colorimetric phenol-hypochlorite method (Koroleff 1983). Concentrations of nitrate, nitrite, and phosphate were measured using a Lachat QuikChem 8500 autoanalyzer (Parsons et al. 1984). Nutrient data are reported as the mean ± the standard error of triplicate samples (Table 2).

Spatial and temporal calculations

A first order regional and pan-Arctic estimate of the amount of nitrogen that may be released via marine N_2 fixation was calculated using the total volume in the upper 50 m of the ice-free water column, integrated over the summer season (June–September), then multiplying that volume by the range of N_2 fixation rates observed in this and the Blais et al. (2012) studies. The extrapolation of the total nitrogen fixed under ice-free conditions between June and September is done for three domains: western Arctic shelves, pan Arctic continental shelves, and the entire Arctic Ocean. The western Arctic shelf is specified because the N_2 fixation rates reported here and in Blais et al. (2012), encompass the area between Baffin Bay on the northeastern boundary and the Chukchi Sea on the northwestern boundary (Fig. 1). For the purpose of this study, the western Arctic shelf was defined as bodies of water in the longitudinal range between 175°E and 45°W with water column depths shallower than 200 m and deeper than 1 m. Generally, this covers the Chukchi Sea, Beaufort shelves, Canadian Arctic Archipelago, the shelves surrounding Baffin Bay, and northern portion of the Labrador Sea. The Arctic continental shelves domain was defined as bodies of water that are not rivers or lakes north of latitude 64°N and with a water column depth shallower than 200 m and deeper than 1 m. The Arctic Ocean domain was defined as bodies of water that are not rivers or lakes north of latitude 64°N and with a water column depth of 1 m or greater. The 1 m shallow depth cut off was implemented to eliminate regions that are strongly wave-influenced or very shallow tidal systems as measurements used in this study were not made in those types of environments.

For the volumetric calculations of seasonally ice-free waters, we used the 30 arcsec resolution IBCAO3 bathymetric data (Jakobsson et al. 2012) and Multisensor Analyzed Sea Ice Extent (MASIE) Northern Hemisphere data published by the National Snow and Ice Data Center (Fetterer et al. 2010). The MASIE sea ice cover data has a constant horizontal resolution of 4 km, with each grid covering an area of 16 km^2. The climatological open water extent is calculated using MASIE data from 2006 to 2016.

Table 2. Chemical and N_2 fixation rate data.

Station	NH_4^+ (μmol N L^{-1})	NO_3^- (μmol N L^{-1})	PO_4^- (μmol P L^{-1})	N_2 fixation rate (nmol N L^{-1} d^{-1})		> 3 μm:
				Whole	> 3 μm	Whole (%)
Marine						
1	1.55 ± 0.07	0.45 ± 0.00	0.55 ± 0.01	5.7 ± 1.7	1.6 ± 0.3	34
2	0.59 ± 0.00	0.32 ± 0.00	0.51 ± 0.00	17.2 ± 7.0	12.6 ± 5.0	84
3	0.47 ± 0.02	0.33 ± 0.00	0.47 ± 0.00	3.5 ± 0.2	1.1 ± 0.2	31
4	0.59 ± 0.03	0.29 ± 0.00	0.55 ± 0.00	4.4 ± 1.2	1.7 ± 0.5	40
8	0.22 ± 0.00	0.30 ± 0.00	0.39 ± 0.00	7.7 ± 2.1	1.9 ± 0.4	25
Average	***0.68 ± 0.12***	***0.34 ± 0.02***	***0.49 ± 0.01***	***7.7 ± 1.8***	***3.8 ± 1.5***	***43***
Estuarine						
5	0.14 ± 0.01	0.22 ± 0.01	0.17 ± 0.00	4.9 ± 0.4	2.6 ± 0.5	52
6	0.11 ± 0.00	0.59 ± 0.00	0.17 ± 0.01	5.4 ± 0.3	5.1 ± 0.6	96
7	0.10 ± 0.00	0.21 ± 0.00	0.45 ± 0.00	5.6 ± 0.6	3.1 ± 0.2	57
Average	***0.12 ± 0.01***	***0.34 ± 0.06***	***0.22 ± 0.05***	***5.3 ± 0.2***	***3.6 ± 0.4***	***68***

Station numbers correspond to site locations described in Table 1. Samples were collected 15–20 August 2011. Data represent the mean ± the standard error of triplicate samples.

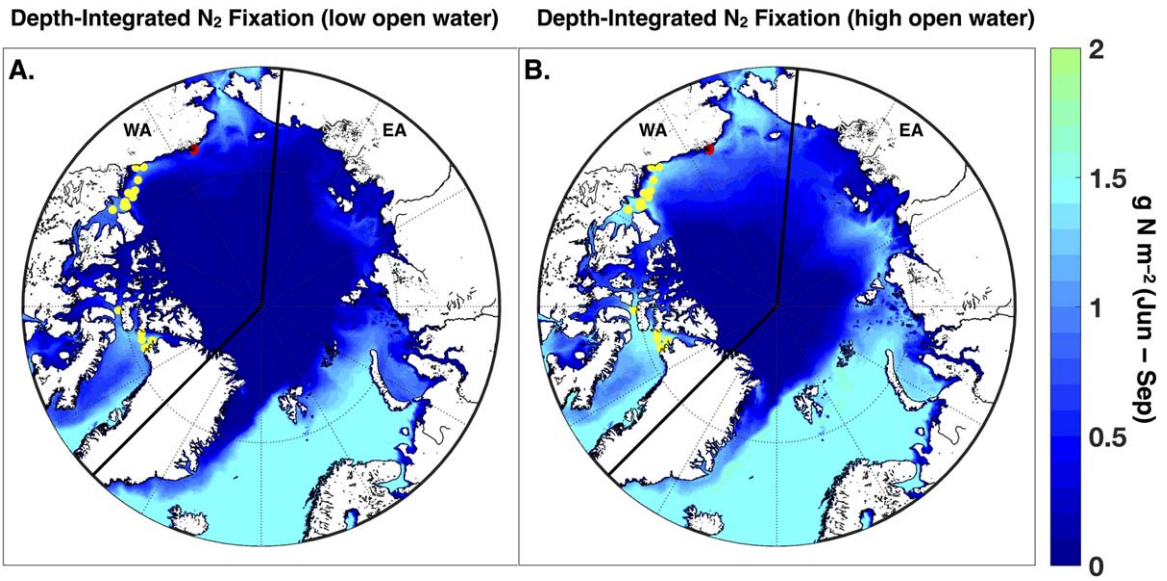

Fig. 1. Summer depth-integrated contribution in the Arctic Ocean under low and high open water conditions. The maps depict the time averaged, depth-integrated nitrogen fixed per unit area during the summer months in the Arctic Ocean. The calculation is based on the number of open water days for each location in the Arctic Ocean and the integration is down to a maximum depth of 50 m which includes both the upper mixed layer as well as the seasonal pynocline. The maps depict the amount of nitrogen contributed to the Arctic Ocean between June and September under (A) low (1 standard deviation below the mean condition) and (B) high (1 standard deviation above the mean condition) open water scenarios. The MASIE ice extent data used in the calculation is from June to September (2006–2016). A solid black line bisecting the maps delineates the Western Arctic (WA) longitudinal range used in this study. The remaining Eastern Arctic sector is identified as EA. Sites listed in Table 1 are depicted by red circles and sites sampled by Blais et al. (2012) are depicted by yellow circles. These points provide the spatial range of sites with measured N_2-fixation rates and the data used in the extrapolations presented in Table 3.

For each of the three domains that we described above (western Arctic shelves, pan-Arctic shelves, and whole Arctic Ocean), the total amount of nitrogen fixed each year was calculated by integrating, in space and time, the measured N_2 fixation rates over the water column depth down to a maximum depth of 50 m for ice-free days from 01 June to 30 September. These restrictions in time and depth are based on the conditions under which the observations were made (Blais et al. 2012; this study) and our assumptions about the depth range over which the measurements may be valid. All

available measurements were collected under ice-free conditions, within the seasonal surface mixed layer. For the purpose of this extrapolation, we have assumed that rates are constant above 50 m depth because the goal was to determine the maximum contribution potential. Admittedly this may be a source of error if the profile of N_2 fixation rates is a function of depth. Recent *nifH* studies indicate that the majority of Arctic marine diazotrophs are heterotrophic (~ 92%; Fernández-Méndez et al. 2016) and thus may not be directly dependent on light for energy. More studies are required to understand the physiological limitations of Arctic pelagic diazotrophs.

To estimate the potential range of uncertainty introduced by variability in seasonal sea ice cover from year to year, we also calculated the standard deviation of the extent of the seasonal open water area from 2006 to 2016. The standard deviation of the sea ice cover map is then added and subtracted from the mean sea ice cover map, then integrated with the measured N_2 fixation rates to obtain estimates of total fixed nitrogen for both "high" and "low" open water years. Maps of the high (mean + standard deviation) and low (mean − standard deviation) open water days for the entire Arctic are shown in Fig. 1.

Table 3. Contribution of marine N_2 fixation to local and global nitrogen budgets.

	Ice-free shelves western Arctic	Ice-free shelves entire Arctic
Tg N yr^{-1}	$\leq 1.2 \pm 0.3$	$\leq 3.5 \pm 0.7$
% Annual global N_2 fixation	≤0.9	≤2.7
% Arctic denitrification deficit	0.01–9.3	0.02–27.1

Amount of nitrogen contributed by Arctic N_2 fixation and its potential proportional contributions to annual global N_2 fixation (estimated at 130 Tg N yr^{-1}; Eugster and Gruber 2012) and the Arctic denitrification deficit (estimated at 13 Tg N yr^{-1}; Chang and Devol 2009) based on two scenarios from June to September: (1) N_2 fixation limited to ice-free shelves in the western Arctic Ocean (1.74 million km^2) and (2) N_2 fixation present on all ice-free shelves in the entire Arctic Ocean (4.91 million km^2). The Arctic Ocean is defined as bodies of water that are not rivers or lakes north of 64°N with a water column depth of 1 m or greater. Continental shelves are defined as bodies of water that are not rivers or lakes between 1 m and 200 m in depth; the western Arctic shelf is defined in the longitude range between 175°E and 45°W with water between 1 m and 200 m in depth. The total integrated depth was 50 m or less depending on total water column depth. The ranges reported here are based on the range of marine N_2 fixation rates (0.01–17.15 nmol N L^{-1} d^{-1}) reported by Blais et al. (2012) and this study.

Results and discussion

Active N_2 fixation was found in the coastal Chukchi Sea near Barrow, Alaska with average rates of 7.7 ± 1.8 nmol N L^{-1} d^{-1} and 5.3 ± 0.2 nmol N L^{-1} d^{-1} at marine and estuarine sites, respectively (Table 2). A comparison of the >3 μm and whole water (>0.7 μm) size fractions revealed that approximately 43% of marine and 68% estuarine diazotrophs were <3 μm in size (Table 2). The rates measured in the Chukchi Sea are higher than rates (0.01 ± 0.01 nmol N L^{-1} d^{-1} to 4.45 ± 0.23 nmol N L^{-1} d^{-1}) measured in the Canadian Arctic (Blais et al. 2012). The N_2 fixation rates reported here and in Blais et al. (2012), encompass the area between Baffin Bay on the northeastern boundary and the Chukchi Sea on the northwestern boundary. Assuming uniform N_2 fixation rates in the upper 50 m of the water column, representing the surface mixed layer and the seasonal pycnocline, we estimate that N_2 fixation on the western Arctic shelves can provide 0.01–0.89 mmol N m^{-2} d^{-1}, which would contribute up to 1.2 Tg N yr^{-1} (Table 3). However, most Arctic coastal regions fall within the comparatively wide ranges of temperature (−1.2°C to 19.4°C), salinity (4.4–32.4), and nutrient concentrations ($<$ 0.03–6.92 μmol nitrate L^{-1} and 0.17–1.25 μmol phosphate L^{-1}) where N_2 fixation was observed in the western Arctic (Blais et al. 2012; this study), which suggests that like the conditions under which it was found, N_2 fixation may be more widespread in the Arctic Ocean. Indeed recent investigations using *nifH* gene sequencing found a diverse array of diazotrophs throughout the central Arctic and Eurasian basin (Fernández-Méndez et al. 2016).

The Arctic Ocean covers 20% of the continental shelf area in the global ocean. If N_2 fixation occurs across all Arctic shelves, the total amount of N_2 fixed would increase to 3.5 Tg N yr^{-1} or 2.7% of global N_2 fixation, based on the current estimate of global marine N_2 fixation of 130 Tg N yr^{-1} (Table 3; Eugster and Gruber 2012). Taking this analysis to the extreme, if N_2 fixation is widespread across the entire ice-free Arctic, Arctic marine N_2 fixation could contribute as much as 9.2 Tg N yr^{-1} or 7.1% of global marine N_2 fixation in the ice-free summer months.

We note that the estimates described here are based on *net* N_2 fixation rates. Numerous studies have shown that some diazotrophs release upwards of 50% of recently fixed nitrogen as ammonium or dissolved organic nitrogen (e.g., Glibert and Bronk 1994; Benavides et al. 2013). Therefore, the amount of nitrogen released into the ice-free Arctic Ocean is likely more than our reported rates imply.

We also estimated the variability introduced by variation in annual sea ice cover during the summer months. For the entire Arctic Ocean, using the upper range of the observed N_2 fixation rates (17.2 nmol N L^{-1} d^{-1}), the 1 sigma anomaly in total fixed N_2 is 1.7 Tg N yr^{-1} or 18% of the estimate based on average summer sea ice cover. For just the Arctic shelf regions, the 1 sigma anomaly is 0.71 Tg N yr^{-1} or 21% of the average, and for the western Arctic only shelf regions, the 1 sigma anomaly is 0.26 Tg N yr^{-1} or 22% of the average.

The presence of active N_2 fixation in the Arctic is relevant to outstanding questions of whether the nitrogen budget of the Arctic is balanced. Although some estimates suggest that the annual Arctic nitrogen budget is balanced (Torres-Valdés et al. 2013), measured rates of denitrification on the Arctic shelves are high and produce an estimated nitrogen deficit of 13 Tg N yr^{-1} (Chang and Devol 2009), with indirect rate calculations generating higher estimates (Mills et al. 2015). This implies that there is a currently unquantified source of nitrogen to the Arctic Ocean. Although some studies have concluded that N_2 fixation is too small to offset any appreciable portion of denitrification (Blais et al. 2012; Mills et al. 2015), our data suggests otherwise. Comparing the estimated denitrification deficit with our N_2 fixation estimates described above, N_2 fixation within the ice-free western Arctic would offset up to 9.3% of the estimated 13 Tg N yr^{-1} Arctic denitrification deficit (Chang and Devol 2009). This increases to 27.1% if N_2 fixation is occurring across all ice-free Arctic shelves. Again, taking the extrapolation to the extreme, 70.7% of the Arctic denitrification deficit could be offset by nitrogen fixation if it is active across the entire seasonally ice-free Arctic Ocean (Table 3).

If we consider just the Chukchi shelf, N_2 fixation could offset 40% of the denitrification based on direct measurements of denitrification (average measured rate of 0.96 mmol N m^{-2} d^{-1}; Devol et al. 1997; Chang and Devol 2009) with the average N_2 fixation rates observed at our marine sites (0.39 ± 0.09 mmol N m^{-2} d^{-1}). Although we constrain the depth used for calculations to 50 m based on current measurements (Blais et al. 2012; this study), which are limited to the surface mixed layer, N_2 fixation has now been found at sub-euphotic depths (Hamersley et al. 2011; Jayakumar et al. 2012; Rahav et al. 2013). Sub-euphotic N_2 fixation would greatly increase the potential contribution of N_2 fixation to the Arctic as would sea-ice and under-ice N_2 fixation (Fernández-Méndez et al. 2016). We have also not included N_2 fixation in Arctic marine sediments in our estimates, which would add a small (0.001–0.02 mmol N m^{-2} d^{-1}) additional source of fixed nitrogen (Knowles and Wishart 1977; Haines et al. 1981; Gihring et al. 2010). These additional sources would further increase the Arctic's potential as an important source of newly fixed nitrogen and underscore the need for more comprehensive studies investigating the rate of N_2 fixation throughout the Arctic Ocean over broader spatial and temporal scales.

From a global change perspective, the most pressing question is whether N_2 fixation has always occurred in the Arctic or whether it is an emerging phenomenon. The Chukchi Sea has warmed by 0.6°C and the ice-free period has lengthened by 11 d over the last decade (Stroeve et al. 2014). Earlier assumptions that N_2 fixation was restricted to warm and nutrient impoverished euphotic waters have limited water-column N_2 fixation studies in high-latitude marine systems. However, more than 50 yr ago N_2 fixation rate measurements were made in the lakes, ponds, and coastal ocean around Barrow, Alaska. Though active N_2 fixation was observed in inland waters, no N_2 fixation was detected at any of the estuarine or marine sites (Toetz 1961), including the locations where we found active N_2 fixation in this study. The Toetz (1961) study followed ^{15}N tracer methods described by Dugdale et al. (1959), which differed from the method used in our study. Briefly, the Toetz study aerated samples with an 80 : 20 helium : oxygen mixture under a reduced pressure of 0.8 atm. $^{15}N_2$ gas (95% enriched) was then added to bring the pressure within the sample back up to 1 atm. Any sample with an atom % enrichment higher than 0.386 indicated significant N_2 fixation. With such limited data and a difference in methodological approaches, we cannot say definitively that N_2 fixation is new to the Arctic. However, long-term increases in N_2 fixation have been correlated to changes in climate (Sherwood et al. 2014), and the trend towards greater nitrogen limitation in the region makes N_2 fixation more likely.

If N_2 fixation is increasing in the Arctic, cascading changes in the current nitrogen, carbon, and phosphorus cycles may be significant. Current and projected estimates of carbon sequestration in the Arctic are based on the observations that nitrogen, typically nitrate, limits primary production in this region (Tremblay and Gagnon 2009; Popova et al. 2012). As a source of new nitrogen, N_2 fixation could enhance sequestration of carbon dioxide and should be considered in future estimates. Additionally, the Arctic Ocean is a source of phosphorus to the North Atlantic where it supports primary production and N_2 fixation (Yamamoto-Kawai et al. 2006; Torres-Valdés et al. 2013). If Arctic N_2 fixation is providing an additional source of nitrogen, diazotrophy, and the new biomass that it supports could draw down dissolved phosphorus within the Arctic and ultimately reduce the amount of phosphorus reaching the North Atlantic, potentially altering productivity within that region. Increases in Arctic N_2 fixation could thus profoundly affect areal patterns of productivity within both of these oceans. Decreases in soluble phosphorus, in the absence of nitrate, have been observed in regions of the Canadian Arctic (Simpson et al. 2008; Tremblay et al. 2008), providing evidence that this may already be occurring. Clearly, more studies of N_2 fixation in Arctic waters are needed. The extrapolations provided in this study highlight the potential contributions of Arctic N_2 fixation and the need to better define its current contribution and to determine how this may change in the future.

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