frontiers in Marine Science

OPINION
published: 06 April 2018
doi: 10.3389/fmars.2018.00108

Deep Into Oceanic N_2 Fixation

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Keywords: nitrogen budget, mesopelagic, non-cyanobacterial diazotrophs, *nifH* gene, aphotic layer

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Specialty section:
This article was submitted to Aquatic Microbiology, a section of the journal Frontiers in Marine Science

Received: *06 November 2017*
Accepted: *14 March 2018*
Published: *06 April 2018*

Citation:
Benavides M, Bonnet S, Berman-Frank I and Riemann L (2018) Deep Into Oceanic N_2 Fixation. Front. Mar. Sci. 5:108. doi: 10.3389/fmars.2018.00108

The biological fixation of dinitrogen (N_2) by marine prokaryotes called diazotrophs is the major source of nitrogen to the ocean, estimated at ∼106–120 Tg N y^{-1} (Gruber, 2004; Gruber and Galloway, 2008). This process contributes importantly to sustain primary production and maintain the global nitrogen inventory. The nitrogen reservoir is further controlled by fixed nitrogen loss processes including sediment burial, denitrification, and anammox (Falkowski, 1997), which exceed fixed nitrogen gains through N_2 fixation, leading to an imbalanced global nitrogen budget (Codispoti et al., 2001; Codispoti, 2007; Eugster and Gruber, 2012). Since the early 1970s, diazotrophic activity has been attributed to autotrophic cyanobacteria constrained to the sunlit and oligotrophic layer of the tropical and subtropical oceans (Zehr, 2011). Yet substantial evidence indicates a high diversity and wide distribution of non-cyanobacterial diazotrophs (bacteria and archaea) in the oceans (Zehr et al., 1998, 2000; Farnelid et al., 2011; Bombar et al., 2016; Moisander et al., 2017). These diazotrophs are potentially not constrained by light as are their cyanobacterial counterparts, and have been detected in wide-ranging environments such as nutrient-rich, cold, and/or dark ecosystems including coastal upwelling regions (Sohm et al., 2011), temperate coastal zones (Bentzon-Tilia et al., 2015), and the deep ocean (Hewson et al., 2007; Hamersley et al., 2011).

Stretching the environmental boundaries, beyond those traditionally thought to constrain N2 fixation, will likely impact current estimates of nitrogen input to the global ocean. Extending the latitudinal limits from the tropics and subtropics to temperate waters would already represent a considerable increase in the potentially active N_2 fixation area, but spreading this area vertically to the mesopelagic (200–1,000 m) and bathypelagic (1,000–4,000 m) ocean would be immense. Aphotic N_2 fixation rates are usually low when compared to surface activity (<1 nmol N L^{-1} d^{-1}; see **Table 1** in Moisander et al., 2017) but the volume of the deep ocean is enormous. Consequently, studies comprising both photic and aphotic N_2 fixation measurements report depth-integrated aphotic rates representing 40–95% of the whole water column activity (Bonnet et al., 2013; Rahav et al., 2013; Benavides et al., 2015). Hence, aphotic fixation can account for a significant or even predominant fraction of water column N_2 fixation.

With the mere purpose of illustrating the potential budgetary relevance of the aphotic N_2 fixation to the global fixed nitrogen input, a back-of-the-envelope calculation can be carried out. If we consider a scenario for the mesopelagic zone (where the great majority of published aphotic N_2 fixation measurements were obtained from): taking the lower-end range of aphotic N_2 fixation rates available in the literature (0.01–0.1 nmol N L^{-1} d^{-1}; **Table 1** in Moisander et al., 2017), and the estimated volume of the mesopelagic zone (2.63×10^{17} m^3; Arístegui et al., 2005), mesopelagic N_2 fixation would range between 13 and 134 Tg N y^{-1}. Fixed nitrogen inputs to the ocean include fluvial inputs, atmospheric deposition and biological N_2 fixation, which add up to 187–279 Tg N y^{-1} (**Table 1**). Combining denitrification (including sediment burial) and anammox, fixed nitrogen losses add up to 260–475 Tg N y^{-1} (**Table 1**). Adding mesopelagic N_2 fixation to fixed nitrogen inputs and subtracting losses from gains, we obtain differences ranging from a loss of 183 to a surplus of 114 Tg N y^{-1} (**Table 1**). Despite this extrapolation may be questionable given that data on aphotic N_2 fixation are so sparse that the spatial distribution of mesopelagic N_2 fixation is

TABLE 1 | Global nitrogen budgets and their variability when considering aphotic N_2 fixation.

	Codispoti et al., 2001	Galloway et al., 2004	Gruber, 2008	Jickells et al., 2017
SOURCES				
Pelagic photic N_2 fixation	117	106	120	164
River inputs (DON+PON)	76	48	80	34
Atmospheric deposition	86	33	50	39
Mesopelagic N_2 fixation	13 to 134	13 to 134	13 to 134	13 to 134
Total sources without considering aphotic N_2 fixation	279	187	250	237
Total sources considering aphotic N_2 fixation*	292 to 413	200 to 321	263 to 384	250 to 371
SINKS				
Benthic denitrification	300	206	180	
Water column denitrification	150	116	65	
Sediment burial	25	16	25	
Total sinks	475	338	270	260
Balance without considering aphotic N_2 fixation	−196	−151	−20	−23
Balance considering aphotic N_2 fixation	−183 to −62	−138 to −17	−7 to 114	−10 to 111

*All fluxes are expressed in Tg N y^{-1}. *The range of aphotic N_2 fixation rates considered is 13.45–134.45 Tg N y^{-1}, see the main text.*

unknown, it does illustrate that aphotic N_2 fixation could be important to global nitrogen budget considerations, and thus deep N_2 fixation should be further explored. Considering the stock of fixed nitrogen in the mesopelagic zone (Gruber, 2008) and the range of mesopelagic N_2 fixation rates estimated here (i.e., 13–134 Tg N y^{-1}), N_2 fixed and eventually remineralized to nitrate in the mesopelagic zone would turn over in 4 to 43 y.

The currently available dataset (Table 1 from Moisander et al., 2017, this issue) lacks robustness because (i) the number of measurements is limited and geographically sparse, and (ii) methodological difficulties are entailed in the detection of low N_2 fixation rates. While aphotic N_2 fixation has been consistently reported in several tropical and temperate waters (**Table 1**; Moisander et al., 2017, this issue), it is unknown whether it occurs homogeneously throughout the dark water column or only in micro-niches where suitable conditions are found. Such hospitable niches may comprise aggregates, or organic matter accumulation zones like ecotones, fronts or water mass boundaries (Benavides et al., 2015; Bombar et al., 2016). Only a few studies have documented *nifH* gene expression in aphotic waters (e.g., Jayakumar et al., 2012), and it is debated whether reported abundances of non-cyanobacterial diazotrophs can account for measured rates of N_2 fixation when considering published cell specific rates of cultivated strains (Turk-Kubo et al., 2014; Bentzon-Tilia et al., 2015). This introduces uncertainty to the reliability of measuring especially low N_2 fixation rates (Gradoville et al., 2017), and emphasizes the need for continued refinement of the $^{15}N_2$ incorporation method (Moisander et al., 2017).

In this context, it is pertinent to consider the methodological difficulties encompassed in the detection of low N_2 fixation rates using $^{15}N_2$ as a tracer. The precision of N_2 fixation rates may be affected by (i) a slower than theoretically assumed dissolution of the $^{15}N_2$ bubble in seawater (Mohr et al., 2010; Großkopf et al., 2012), (ii) the contamination of $^{15}N_2$ gas stocks with nitrogenous species other than N_2 (Dabundo et al., 2014), and (iii) failure to measure time zero $\delta^{15}N$ values of the particulate nitrogen pool. As any other tracer method, $^{15}N_2$-based N_2 fixation rates are subject to a number of other sources of error, including variability in incubation and/or filtration time among replicates, sample particle size and its retention in filters varying with filter pore size (Bombar et al., 2018), as well as heterogeneous distribution of particles in Niskin bottles (Suter et al., 2017). Moreover, the vast majority of $^{15}N_2$-based published N_2 fixation measurements report net rates, whereas the leakage of ^{15}N-labeled dissolved organic nitrogen and/or ammonium can be significant in certain cases (e.g., Berthelot et al., 2017).

Most of the compiled aphotic rates (Moisander et al., 2017, this issue) were measured using the bubble method (Montoya et al., 1996), and should be considered as minimum estimates, despite the fact that they were performed in cold waters (typically ∼10°C), which enhances gas dissolution and hence optimizes isotopic equilibrium in seawater samples enriched with $^{15}N_2$ gas. Moreover, the majority of the studies i) used an isotope brand that provides high purity $^{15}N_2$ gas, affecting aphotic N_2 fixation rates by <1% when ^{15}N-labeled nitrogen molecules other than N_2 are taken up (Benavides et al., 2015) and/or ii) provided time zero $\delta^{15}N$ values of the particulate nitrogen pool at each sampling depth, making their results robust (Bonnet et al., 2013; Rahav et al., 2013; Benavides et al., 2015, 2016). Finally, the variability between replicates in all terms included in the N_2 fixation calculation equation (as outlined in Montoya et al., 1996) may throw back minimum quantifiable rates values below estimated aphotic N_2 fixation rates (Gradoville et al., 2017). Propagating errors of the data (Birge, 1940), in five out of the nine aphotic N_2 fixation studies currently available, results in minimum quantifiable rates ranging from 0.01 to 2.7 nmol N L^{-1} d^{-1} (**Table S1**), suggesting

that most of the aphotic N_2 fixation rates published are significant.

The potentially high budgetary significance of aphotic N_2 fixation to the global nitrogen budget calls for further studies that will establish the geographical and temporal distribution of aphotic N_2 fixation and consolidate the volumetric rates published thus far. In future studies, we encourage researchers in the field of marine nitrogen cycling to place emphasis on documenting N_2 fixation in the aphotic ocean and identifying environmental drivers of aphotic N_2 fixation: including oxygen, dissolved organic matter availability and particle colonization (Riemann et al., 2010; Benavides et al., 2015; Bombar et al., 2016). The availability of more data is essential to facilitate modeling and assessment of the distribution and magnitude of aphotic N_2 fixation in the global ocean (i.e., association with water masses, ecotones or density fronts). Eventually, a more comprehensive understanding of the ecophysiology of aphotic N_2 fixers and their contribution to global nitrogen input, will reveal their ecological importance and may help answer such question as what are the evolutionary advantages of the energetically-expensive process of N_2 fixation in an environment rich in dissolved inorganic nitrogen, and how does it affect oceanic carbon sequestration.

AUTHOR CONTRIBUTIONS

MB gathered N_2 fixation rates and made the calculations shown in the tables. MB, IB-F, SB, and LR wrote the article.

ACKNOWLEDGMENTS

MB and LR were supported by grant 6108-00013 from the Danish Council for independent research and by the BONUS BLUEPRINT project receiving funding from BONUS (Art 185) funded jointly from the European Union's Seventh Programme for research, technological development and demonstration and from The Danish Council for Strategic Research (LR).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2018.00108/full#supplementary-material

Table S1 | Error propagation analysis of aphotic N2 fixation rates from various published and unpublished studies.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.