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Questioning High Nitrogen Fixation Rate Measurements in the Southern Ocean

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ARISING FROM T. Shiozaki et al. *Nature Geoscience* https://doi.org/10.1038/s41561-020-00651-7 (2020)

Nitrogen fixation in the global ocean is a microbially mediated process performed by a special class of organisms able to enzymatically reduce dinitrogen gas (N_2) to ammonia to fuel cellular nitrogen (N) demands[1–3]. There now seem to be few ocean habitats wholly unfavourable to at least a subset of N_2-fixing organisms, and, in this context, Shiozaki et al.[4] recently reported substantial nitrogen fixation in one of the coldest and most isolated regions of our planet: the coastal waters near the ice edge of Antarctica[5,6]. The authors speculated that iron additions from melting sea ice fuel N_2 fixation activity of the unicellular cyanobacteria UCYN-A/haptophyte symbiosis and argue that the results suggest that "marine nitrogen fixation is a ubiquitous process in the global ocean, and that UCYN-A is the keystone species for making it possible".

We read this Article with great interest given the very high rates of oceanic N_2 fixation in such a cold (below 0 °C), nitrate-rich (>15 μmol l^{-1}) habitat. We were immediately struck by the magnitude of the rate of 44 nmol N l^{-1} d^{-1} reported at the ice edge[4]. This rate is extraordinarily high; compared with a global compilation by Luo et al.[7], it is in the highest 1% of oceanic rates ever measured (see fig. 1 of Luo et al.[7] and data in ref. [8]). The authors thus stated that the "study sheds light on nitrogen fixation as an alternative source of [reactive N] to support primary production in the Antarctic Ocean" against a backdrop of micromole per litre concentrations of nitrate. This is a claim that compels scrutiny of the data.

Upon examination of data generously shared with us by Shiozaki et al. (not included in the published Article), we were struck by three salient points.

Replicate measurements from Station E were treated differently

The reported rate at Station E of 44 nmol N l^{-1} d^{-1} in sea ice derives from an outlier. All rate determinations yielded low or undetectable rates of N_2 fixation (<2 nmol N l^{-1} d^{-1}) with this one exception. In the methods, it is stated that replicate measurements of pertinent rate-specific terms were conducted[4]. These terms include the inherent nitrogen isotopic composition of particulate material (the atomic per cent (at.%) of particulate nitrogen, PN, before tracer addition, A_{PN-T0}), the N isotopic composition of added tracer (at.% of added $^{15}N_2$, A_{N2}), the incubation period (Δt), and the final N isotopic composition after incubation (at.% of PN at termination of experiment, A_{PN-Tf}), all of which are necessary to accurately quantify the nitrogen fixation rate (NFR)[9], as described in Shiozaki et al.[4] and derived by Montoya et al.[9]:

$$\mathrm{NFR} = \frac{(A_{\mathrm{PN-Tf}} - A_{\mathrm{PN-T0}})}{(A_{\mathrm{N2}} - A_{\mathrm{PN-T0}})} \times \frac{[\mathrm{PN}]}{\Delta t} \quad (1)$$

The data shared with us confirm that all of the above terms were measured (which is commendable, as this is not always done[10]) and rate-specific terms were indeed calculated from replicates at all stations except Station E (the ice-edge station, see Table 1). The reported high rate of 44 nmol N l^{-1} d^{-1} was calculated using one of three replicate measurements of A_{PN-Tf} (0.450%), despite the remaining replicates having a relatively low A_{PN-Tf} (0.368 ± 0.005%). That lone high A_{PN-Tf} value is notably ~6 s.d. from the mean of A_{PN-Tf} values achieved in incubations at other stations where rates were detectable (n = 16, 0.368 ± 0.002%; Table 1). As noted above, rate measurements at all other stations were uniformly low (0.2–1.9 nmol N l^{-1} d^{-1}), and all were estimated from extant replicates at the exclusion of none.

It is not clear why reasonable replicates that were consistent with all other data were discarded, with the exception of one outlier. There are several reasons for a singularly high A_{PN-Tf}, including sample contamination during collection or during storage and processing of samples, carryover in the mass spectrometer or nonlinearities of the mass spectrometer. All of these potential explanations seem more plausible than excluding replicates, and we remain concerned that key underlying data were not presented, and that the implications of the lack of reproducibility at Station E were not discussed. We also note that the at.% increase during the incubations was relatively small, with a median value of 0.0012% (that is, 3‰ versus air), which could easily arise from inherent N cycling (for example, see ref. [11]), rather than the incorporation of the $^{15}N_2$ tracer. Control incubations would have helped address this issue but were not performed. Moreover, the median increase was of the same order as three times the standard deviation of initial A_{PN-T0} values (0.002%), thus at (or below) a standard definition of the limit of detection for this method[10].

Conspicuously low UCYN-A gene abundance

The reported UCYN-A gene abundance at Station E is far too low to account for the corresponding rate of 44 nmol N l^{-1} d^{-1}. Gene abundances of the nitrogenase gene marker for UCYN-A (the proposed driver of these rates) are estimated to be 129 gene copies per litre, and never higher than the maximum of 220 gene copies per litre at any station. If one assumes the highest cell-specific rate measured for this organism (~50 fmol N cell^{-1} d^{-1}, see refs. [12,13]) and assumes one nitrogenase gene copy per cell, one would calculate an equivalent rate of ~0.01 nmol N l^{-1} d^{-1}, which is orders of magnitude too low to account for the reported rate at the ice-edge station. This analysis remains valid even when a recently described correction factor is applied to account for underestimated per-cell rates resulting from isotope dilution effects[14]. Variability in UCYN-A distributions

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Table 1 | Excerpt of data provided on request by Shiozaki et al.[4] - only data the authors deemed as detectable nitrogen fixation rates are shown

Station	NFR (nmol N l^{-1} d^{-1})	UCYN-A (copies l^{-1})	$A_{PN\text{-}T0\text{-}1}$ (at.%)	$A_{PN\text{-}T0\text{-}2}$ (at.%)	$A_{PN\text{-}T0\text{-}3}$ (at.%)	A_{N2} (at.%)	$A_{PN\text{-}TF\text{-}1}$ (at.%)	$A_{PN\text{-}TF\text{-}2}$ (at.%)	$A_{PN\text{-}TF\text{-}3}$ (at.%)	NFR-1 (nmol N l^{-1} d^{-1})	NFR-2 (nmol N l^{-1} d^{-1})	NFR-3 (nmol N l^{-1} d^{-1})
B	0.51	n.d.	0.368	0.368	0.367	3.494	0.369	0.369	0.368	0.50	0.52	0.17
C	0.20	n.d.	0.367			2.024	0.368	0.368		0.17	0.22	
D	0.31	129	0.366			3.489	0.367	0.367		0.35	0.26	
E	44	129	0.368	0.366	0.367	3.583	0.365	0.450	0.372		44.36	1.60
EL	1.01	n.d.	0.366	0.365	0.364	3.489	0.368	0.368	0.371	0.68	0.79	1.56
EL	0.25	126	0.366			2.023	0.368	0.367		0.53	0.40	
EL	1.03	n.d.	0.364			2.025	0.367	0.370		1.15	1.93	

Only data that Shiozaki et al. deemed detectable NFRs are shown. The NFR data correspond to data partially reported in the supplementary information of Shiozaki et al.[4]. All at.% values are truncated to thousandths here but are shown in full precision in the supplementary file provided by the authors. Cells for which no data were available are left empty. The anomalous value that drew our attention was recorded at Station E. PN data were not reported in the ref. [4] or its supplementary information, and hence a minimum quantifiable rate cannot be calculated here.

could potentially explain this mismatch between abundances and rates. However, assuming per-cell rates of ~50 fmol N cell^{-1} d^{-1}, approximately 10^5 UCYN-A cells would be needed per litre to explain this high rate in a single incubation bottle. These 'reasonability' tests further led us to question the validity of the outlier and the exclusion of the replicates in reporting (see fig. 1 in Shiozaki et al.[4] and its supplementary information).

Insufficient support for conclusions

We feel that the authors conclusions that their "results indicate a potential co-relationship between Antarctic nitrogen fixation and the extent and cycle of sea ice, which are likely to be altered by climate change" is an irresponsibly speculative statement given the data on hand. We do not question that UCYN-A nitrogenase genes are present in this region at low levels, and that low rates of N_2 fixation were potentially detected. However, the outlying and unreplicated rate estimate from the ice-edge station does not warrant the level of speculation on ecological drivers of N_2 fixation presented in Shiozaki et al.[4].

It is our hope that by raising these issues we can promote data transparency and a deeper understanding of how to design and interpret $^{15}N_2$ fixation tracer experiments. In our view, the use of a single outlier to weave a narrative is misleading, particularly without reporting the underlying data and a rationale for presentation of the data, and given the ecologically unusual magnitude of this rate compared with decades of historical measurements[7]. As we have argued previously[10], validating the detection of biological N_2 fixation in unexpected realms of the ocean requires both transparency in data reporting and a critical assessment of the data on hand; neither were done thoroughly in our opinion. And so, while we are convinced by Shiozaki et al.[4] that nitrogenase genes of the UCYN-A/haptophyte symbiosis were detectable in the Southern Ocean, where the putative activity of this symbiosis may act as a relatively modest source of reactive nitrogen, the presentation and interpretation of the data are misleading.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41561-021-00873-3.

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References

1. Gruber, N. & Sarmiento, J. L. Global patterns of marine nitrogen fixation and denitrification. *Glob. Biogeochem. Cycles* **11**, 235–266 (1997).
2. Codispoti, L. A. An oceanic fixed nitrogen sink exceeding 400 Tg N a^{-1} vs the concept of homeostasis in the fixed-nitrogen inventory. *Biogeosci. Discuss.* **3**, 1203–1246 (2006).
3. Capone, D. G. & Carpenter, E. J. *Nitrogen Fixation by Marine Cyanobacteria: Historical and Global Perspectives* (Musee Oceanographique, 1999).
4. Shiozaki, T. et al. Biological nitrogen fixation detected under Antarctic sea ice. *Nat. Geosci.* **13**, 729–732 (2020).
5. Zehr, J. P. & Capone, D. G. Changing perspectives in marine nitrogen fixation. *Science* https://www.science.org/doi/10.1126/science.aay9514 (2020).
6. Tang, W., Li, Z. & Cassar, N. Machine learning estimates of global marine nitrogen fixation. *J. Geophys. Res. Biogeosci.* **124**, 717–730 (2019).
7. Luo, Y.-W. et al. Database of diazotrophs in global ocean: abundance, biomass and nitrogen fixation rates. *Earth Syst. Sci. Data* **4**, 47–73 (2012).
8. Luo, Y. et al. Global distributions of diazotrophs abundance, biomass and nitrogen fixation rates: gridded data product (NetCDF): contribution to the MAREDAT World Ocean Atlas of Plankton Functional Types. *PANGAEA* https://doi.pangaea.de/10.1594/PANGAEA.774851 (2012).
9. Montoya, J. P., Voss, M., Kahler, P. & Capone, D. G. A simple high-sensitivity tracer assay for N_2 fixation. *Appl. Environ. Microbiol.* **62**, 986–993 (1996).
10. White, A. E. et al. A critical review of the $^{15}N_2$ tracer method to measure diazotrophic production in pelagic ecosystems. *Limnol. Oceanogr. Methods* **18**, 129–147 (2020).
11. Fripiat, F. et al. New insights into sea ice nitrogen biogeochemical dynamics from the nitrogen isotopes. *Glob. Biogeochem. Cycles* **28**, 115–130 (2014).
12. Harding, K. et al. Symbiotic unicellular cyanobacteria fix nitrogen in the Arctic Ocean. *Proc. Natl Acad. Sci. USA* **115**, 13371–13375 (2018).
13. Krupke, A. et al. The effect of nutrients on carbon and nitrogen fixation by the UCYN-A–haptophyte symbiosis. *ISME J.* **9**, 1635–1647 (2015).
14. Meyer, N. R., Fortney, J. L. & Dekas, A. E. NanoSIMS sample preparation decreases isotope enrichment: magnitude, variability and implications for single-cell rates of microbial activity. *Environ. Microbiol.* https://sfamjournals.onlinelibrary.wiley.com/doi/abs/10.1111/1462-2920.15264 (2020).

Data availability

All data reported here are in raw form in Table 1, as shared by T. Shiozaki. Data are largely reproduced from the Supplementary Data of Shiozaki et al.[4]

Author contributions

A.E.W. drafted the initial correspondence and text; all authors contributed to the writing and editing and have approved the submitted version.

Competing interests

The authors declare no competing interests.

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