We have taken all the comments of the reviewers into account in the revision; replies to each of the comments are provided below in blue fonts.

Reviewer #1:

Shao et al. present an update of the diazotroph database published in 2012 https://essd.copernicus.org/articles/4/47/2012/

The new version adds up data published between 2012 and 2023, including volumetric and depth-integrated N_2 fixation rates, diazotroph microscope counts and nifH gene counts. This new version also discusses microscope-nifH count comparisons. While this update is valuable for the community as a tool for comparison and contextualization of diazotrophy studies, it fails to account for many diazotrophy studies published between 2012 and 2023. The text has several misinterpretations that need correction. The new version also includes N2 fixation rates proxied with other methods (ARA). I think this is a major problem, since these rates are not currently solidly comparable and downplay the robustness of the database. The manuscript also eliminates nifH gene counts from non-cyanobacterial diazotrophs (NCDs), which is another major issue since NCDs are considered to be outnumber cyanobacterial diazotrophs in the ocean. Finally, the diazotroph microscopy count versus nifH gene count conversion discussion does not seem appropriate here, since very few of the papers listed have compared these approaches on a same given sample, and the issue has been discussed thoroughly in other publications by specialists.
In all, while I acknowledge the effort and usefulness of this manuscript, I advise major revisions as detailed in the comments below.

Response: We thank the reviewer for very constructive and thorough comments. We particularly appreciate the reviewer's suggestion to include PIs of the data sources as coauthors, and we have adopted this suggestion. We are pleased to report that more than 80 PIs have agreed to join this collaborative effort. By doing so, we believe that this paper will not only showcase the significant impact of collective research efforts in N_2 fixation, but also the completeness of the database and the quality of the paper can be improved substantially. The new coauthors have started to identify missing datasets and to provide additional comments that will further enhance the quality of the paper. Other general comments have also been addressed:

(1) We have decided not to include the ARA-based data in estimating the global N_2 fixation rate, but we have included them in the database for those who are interested in using them.

(2) The NCD data have been added to the database as an additional spreadsheet.

(3) We have decided to keep the comparison of *nifH* gene copies and diazotrophic cell counts in the paper, and we invite you to review our response to the related comments.

Once again, we thank the reviewer for their valuable feedback, which has helped us to improve the quality of our manuscript.

L28: N_2 gas is not inert to diazotrophs.

Response: We have changed the text to "Dinitrogen (N_2) fixation is a process carried out by a group of microorganisms known as diazotrophs. They are capable of converting the N_2 gas, which is not usable by most organisms, into bioavailable nitrogen (N)".

L31: The balance between N loss/gains in the ETSP has been widely demonstrated to be false in several publications after that of Deutsch et al., see for example (Knapp et al. 2016; Bonnet et al. 2017).

Response: Thanks for the comment. Here, we tried to introduce the general function of nitrogen fixation on the global scale. To avoid misleading, we have revised the text to "and contributes to compensate N loss mechanisms such as denitrification and anammox".

L35: Only cyanobacterial diazotrophs can be confidently counted by microscopy.

Response: The text has been revised as "Diazotrophic abundance can be estimated from their *nifH* gene copies using qPCR assays (Church et al., 2005). The abundance of some cyanobacterial diazotrophs can also be directly obtained by counting their cells using microscopes."

L36: "NifH gene copies"

Response: Corrected.

L40: This issue has been thoroughly discussed in (Gradoville et al. 2022), validating the use of nifH gene counts as a means to quantify diazotrophs.

Response: Gradoville et al. (2022) is a regional study in which all the diazotrophs were sampled in two cruises (June 2017 and April 2018) near the Hawaii Islands or along a transect of several hundred kilometers at fixed depths (5 m and 15 m, respectively). Gradoville et al. (2022) described their study as: "... expeditions which each spanned >200 km (Fig. 1). While limited, this reflects the most geographically extensive field comparison of *nif*H:cell among taxa to date."

Hence, although Gradoville et al. (2022) has shown a strong relationship between *nifH* gene counts and diazotrophic (*Crocosphaera*, *Richelia* and *Calothrix*) abundances, it has not sufficiently indicated that this finding is applicable to diazotrophs sampled in other regions or time. Gradoville et al. (2022) partly attributed the large varieties of *nifH*:cell found in Sargent et al., (2016) and White et al. (2018) to potential methodological issues; but they also concluded that "*nif*H is a useful yet imperfect abundance proxy" and urged "future studies report *nif*H:cell and explore the mechanisms controlling this ratio".

We have therefore decided to keep this sentence in our revised manuscript, followed by an introduction of Gradoville et al. (2022): "However, a recent regional study spanning over 200 km in the North Pacific Subtropical Gyre has revealed a robust and statistically significant

correlation between the abundance of the *nifH* gene and cell counts in the UCYN group B (*Crocosphaera*) and heterocystous cyanobacteria (*Richelia* and *Calothrix*) but not in *Trichodesmium* (Gradoville et al., 2022). Nevertheless, the previously observed wide range of *nif*H:cell ratios could be partly attributed to methodological imperfections (Gradoville et al., 2022), which highlights the need for further investigations in this issue."

L42-47: Other sources of unbalance should be briefly mentioned here.

Response: Thanks for the suggestion. The text has been revised as:

"One of possible reasons for this imbalance is inaccurate estimation of global marine N_2 fixation due to limited spatio-temporal coverage of measurements and questionable N_2 fixation assays (White et al., 2020). Another possible reason is the limited knowledge of ecological niches of N_2 fixation. Over the last decade, marine habitats beyond the traditionally recognized, well-stratified oligotrophic tropical and subtropical oceans, such as aphotic waters (Bonnet et al., 2013), coastal areas (Tang et al., 2020), subpolar (Sato et al., 2021; Shiozaki et al., 2018) and even polar regions (Shiozaki et al., 2020; Harding et al., 2018), have demonstrated substantial N_2 fixation. Other studies have also suggested that non-cyanobacterial diazotrophs (NCDs) may be significant contributors to marine N_2 fixation (Shiozaki et al., 2014; Geisler et al., 2020; Turk-Kubo et al., 2022) and may occupy different niches from cyanobacterial diazotrophs (Shao and Luo, 2022)."

L50: Diazotroph activity was there before, it is our notion of them that increases, the data available.

Response: Thanks for the comment. The text has been revised and combined into the above paragraph (see response immediately above).

L56-57: I don't think that the dataset assembled here covers enough studies comparing microscopy and nifH based comparisons, and I strongly recommend removing this sentence and section 4.2 from the manuscript.

Response: We thank the reviewer for the comment. We believe it is necessary to include the comparisons of cell counts and *nifH* gene copies in the manuscript for two reasons. First, as discussed in our response to the above comment, the relationship between cell counts and *nifH* gene copies is still debatable. Second, a large number of measurements have been conducted on diazotrophic cell counts and *nifH* gene copies, particularly those of *Trichodesmium* and *Richelia* (Fig 11, n = 2377 vs. 3070 for *Trichodesmium*; 898 vs. 1771 for *Richelia*, with more data to be added in the revised manuscript). These comparisons can reveal the overall distributions of cell counts and *nifH* gene copies in specific diazotrophic groups, providing another angle as a meta-analysis that complements previous studies that have directly compared

nifH and cell counts using a limited number of samples.

We have also slightly revised the sentence to more accurately describe our analyses: "We also analyzed the discrepancy in N_2 fixation assays and compared the observed ranges of *nifH* gene copies and diazotrophic cell abundance using the data available in the database."

L61: The N_2 fixation rates from Tang et al. 2019 are based on an ARA-$^{15}N_2$ fixation comparison including only 8 data points. This is not robust enough to provide a reliable comparison and downplays the robustness of the $^{15}N_2$-based rates dataset collected here. I strongly recommend removing these from the database and derived basin-scale and global calculations. These may be mentioned as discussion and the Tang paper cited, but not included for quantitative purposes.

Response: Thanks for the comment. Here, we referred to a diazotroph dataset compiled by Tang et al. (2019) and Tang and Cassar (2019) with historical measurements in 2012-2018. There were other in-situ N_2 fixation rates (15 $^{15}N_2$-based and 85 ARA-based measurements) measured by Cassar/Tang's own group (Tang et al., 2019; Tang et al., 2020); these data were also collected into our database. The derived N_2 fixation rates in Tang et al. (2019) were not collected into our database.

We reconsidered ARA-based measurements of N_2 fixation rates and agreed with the reviewer. We have decided not to include the ARA-based data in estimating the global N_2 fixation rate, while keep them in the database for those who are interested in using them.

L72: Removing NCDs is an error in my opinion. NCDs have recurrently been shown to be dominant in the ocean (Farnelid et al. 2011; Delmont et al. 2018, 2021; Riemann, Farnelid, and Steward 2010) and may impact N cycling decisively (Riemann et al. 2022; Turk-Kubo et al. 2022). I strongly recommend that any nifH gene counts of NCDs are added. The previous database included Gamma A and Cluster III. I don't see a solid reason to remove NCDs from the database at this stage, as evidence of their importance increases.

Response: Thanks for the comment. One of the reasons why we did not include NCD data was the existence of a comprehensive NCD dataset compiled by Turk-Kubo et al. (2022). We have now obtained the agreement from Turk-Kubo to include her NCD dataset in the database (she has agreed to be a coauthor of the revised manuscript). Additional NCD data published in several recent studies have also been added to the revised database. We then accordingly changed the sentence to:

"A recently compiled NCD dataset (Turk-Kubo et al., 2022) including 7385 *nifH* gene copies of mostly studied phylotype Gamma A (Shao and Luo, 2022) and other phylotypes, and several recently published NCD data (Bonnet et al., 2023; Sato et al., 2022; Reeder et al., 2022; Turk-Kubo et al., 2021; Wen et al., 2022; Moore et al., 2018), were included in the database."

Line 82: Group-specific N_2 fixation rates can only be estimated using single-cell approaches. I'm not sure what approach was followed here to derive specific rates, but these can certainly not be estimated with the data collected here. I would rather recommend the authors to collect all *Trichodesmium*, UCYN-B, DDAs and UCYN-A single-cell rates published, which would be very helpful for the community. See for instance (Foster, Sztejrenszus, and Kuypers 2013;

Foster et al. 2011; Benavides et al. 2017; Bonnet et al. 2016; Filella et al. 2022; Krupke et al. 2015; K. Harding et al. 2018; Mills et al. 2020; K. J. Harding et al. 2022; Benavides et al. 2022).

Response: Thanks for the comment and we are sorry for the confusing. The "different groups" here referred to different size groups. In the original database of 2012, N_2 fixation rates in samples with size >10 μm were assigned to *Trichodesmium* and those of smaller sizes were assigned to UCYN. In the revised database, we have corrected and reported them as N_2 fixation rates of size groups > 10 μm and < 10 μm, respectively. In some studies, N_2 fixation rates of *Trichodesmium* and heterocystous cyanobacteria were estimated by multiplying their cell abundance with their cell-specific N_2 fixation rates; we also collected these diazotrophic group-specific data into the new version of the database.

We agree with the reviewer that the cell-specific N_2 fixation rates are important and valuable. The cell-specific N_2 fixation rates recommended by the reviewer have been collected into the revised database as a new spreadsheet.

The paragraph has been revised as:

"Same as in the original database, the diazotrophic abundance data in Version 2 were grouped into three taxonomic categories: *Trichodesmium*, UCYN, and heterocystous cyanobacteria. The UCYN abundance data were further grouped into UCYN-A, UCYN-B, and UCYN-C, while heterocystous cyanobacterial abundance was grouped into *Richelia* and *Calothrix*. N_2 fixation rates were measured for whole seawater samples, for different size groups (> 10 μm and < 10 μm), or specifically for *Trichodesmium* and heterocystous cyanobacteria. When whole-water N_2 fixation rates were not reported, total N_2 fixation rates were calculated as the sum of the N_2 fixation rates of available groups. Additionally, 392 data of cell-specific N_2 fixation rates were also collected to Version 2."

Tables 2 and 4: Many studies are missing in this table, some include (Benavides et al. 2014, 2021; Saulia et al. 2020; Henke et al. 2018; Bonnet et al. 2018; Gradoville et al. 2017; Moreira-Coello et al. 2017; Wilson et al. 2019). Also, in the table some studies are listed as not including counts of some diazotrophs, which needs correction (e.g. Bombar 2011 and Bonnet 2015, 2019 did have qPCR counts). Please revise all these publications thoroughly and correct accordingly.

Response: We thank the reviewer for identifying missing datasets and parameters. We have checked datasets suggested by the reviewer, and have added those parameters collected by this database. We have identified more missing datasets and have added them to the revised database. The new datasets added in the revised database are as follows:

N_2 fixation rates:

(1) Benavides et al. (2014), Journal of Marine Systems
(2) Benavides et al. (2017), Scientific Reports
(3) Benavides et al. (2021), ISME Comm.
(4) Benavides et al. (2022), ISME J.
(5) Bonnet et al. (2023), ISME J.
(6) Cerdan-Garcia et al. (2022), ISME J.
(7) Foster et al. (2022) , ISME J.
(8) Gradoville et al. (2017), Limnol. & Oceanogr.

(9) Harding et al. (2022), Nature Comm.
(10) Jiang et al. (2023), J. Geophy. Res.
(11) Kittu et al. (2023), Gobal Biogeochemical Cycles
(12) Landou et al. (2023), Limnol. & Oceanogr.
(13) Messer et al. (2021), PeerJ
(14) Mills et al. (2020), ISME J.
(15) Moreira-Coello et al. (2017), Front. in Mar. Sci.
(16) Rase et al. (2013), Marine Ecology Progress Series
(17) Sato et al. (2022), JGR Biogeosciences
(18) Saulia et al. (2020), Front. in Mar. Sci.
(19) Selden et al. (2021), Limnol. & Oceanogr.
(20) Singh et al. (2017), Geophy. Res. Let.
(21) Singh et al. (2019), Continental Shelf Res.
(22) Turk-Kubo et al. (2021), ISME Comm.

Cell counts:
(1) Estrada et al. (2016), PLOS one
(2) Mompean et al. (2016), J. of Phyto. Res.
(3) Tenório et al, (2018), Aquat. Micro. Ecol.

NifH gene copies:
(1) Bonnet et al. (2023), ISME J.
(2) Cerdan-Garcia et al., (2021), ISME J.
(3) Jiang et al. (2023) JGR Biogeosciences
(4) Bonnet et al. (2015), Gobal Biogeochemical Cycles
(5) Cabello et al. (2020), Journal of Phycology
(6) Messer et al. (2021), PeerJ
(7) Mills et al. (2020), ISME J.
(8) Sato et al. (2022), J. Geophy. Res.
(9) Saulia et al. (2020), Front. in Mar. Sci.
(10) Selden et al. (2021), Limnol. & Oceanogr.
(11) Selden et al. (2022), Front. in Mar. Sci.
(12) Turk-Kubo et al. (2021), ISME Comm.

L104: The ARA method is rarely used nowadays

Response: The reviewer was correct. We have revised the texts to:
"The commonly used methods for marine N_2 fixation rates include $^{15}N_2$ assimilation and acetylene reduction assay (Mohr et al., 2010; Montoya et al., 1996). In the last decade, most samples were measured using $^{15}N_2$ assimilation methods."

L106-107: The ARA to N_2 fixation ratio is highly variable (Mulholland et al. 2006; Benavides et al. 2011; Wilson et al. 2012)

Response: Thanks for this comment. We have added the previously reported range of the conversion factor between acetylene reduction and N_2 fixation:

"The acetylene reduction assay estimates gross N_2 fixation rates indirectly from the reduction of acetylene to ethylene. Theoretical conversion factors of 3:1 or 4:1 has been used to convert acetylene reduction rates to N_2 fixation rates (Postgate, 1998; Capone, 1993; Wilson et al., 2012). However, a wide range of conversion factors from 0.93 to 56 has been reported (e.g., Mague et al., 1974; Graham et al., 1980; Montoya et al., 1996; Capone et al., 2005; Wilson et al., 2012)."

L110: Many other factors affect this difference, including acetylene gas impurity, Bunsen dissolution coefficient, etc.

L112: This is not true. The $^{15}N_2$ method is much more sensitive, does not require biomass preconcentration (biomass is concentrated during filtration, after the incubation), and requires longer incubations for enough tracer to be detectable in biomass. ARA is usually done in 3-4 h incubations and requires biomass pre-concentration to reach detectable signal (Staal et al. 2007; Benavides et al. 2011).

Response: We thank the reviewer for the above two related comments regarding comparing $^{15}N_2$ assimilation and the acetylene reduction assay. We have incorporated the reviewer's comments and corrections and modified the texts as follows:

"Overall, the $^{15}N_2$ assimilation method only measures the fixed N in particulate forms and ignores the N that is fixed but then excreted by diazotrophs during incubation, which, however, can theoretically be counted by the acetylene reduction assays (Mulholland, 2007). Compared to the $^{15}N_2$ assimilation method, the acetylene reduction assay is easier to conduct and needs a shorter incubation time. However, in addition to the uncertainty in converting ethylene production to N_2 fixation, the purity of acetylene gas, trace ethylene contamination and the Bunsen gas solubility coefficient of produced ethylene can also affect the accuracy of estimating N_2 fixation rates (Giller, 1987; Hardy et al., 1973; Flett et al., 1976; Hyman and Arp, 1987). Acetylene used in the assay can even impact the metabolic activities of diazotrophs (Giller, 1987; Hardy et al., 1973; Flett et al., 1976). Moreover, the acetylene reduction assays need to pre-concentrate cells for signal detection when diazotrophic biomass is low, which can damage cells during filtration and cause underestimated N_2 fixation rates (e.g., Capone et al., 2005; Staal et al., 2007; Bhavya et al., 2019; Barthel K-G, 1989). In contrast, the $^{15}N_2$ assimilation method has a higher sensitivity and does not require the cell pre-concentration before incubations."

L120: Wannicke et al. say the opposite of Mohr and Grosskopf.

L123: What White et al. say is that the bubble release method is the most reliable and recommended by the diazotroph research community, with the elimination of rate underestimation benefits overcoming the very unlikely burdens of contamination. This should be corrected in L274-275 as well.

Response: Thanks for pointing out these two mistakes. We have carefully revised whole section:

"The original $^{15}N_2$ assimilation method involved bubbling $^{15}N_2$-labelled gas. However, this method was later found to be inadequate for reaching complete solubility equilibrium over a

short incubation time, resulting in significant underestimations of N_2 fixation rates (Mohr et al., 2010; Großkopf et al., 2012). To address this issue, the $^{15}N_2$ dissolution method was employed, which involved pre-preparing $^{15}N_2$-enriched seawater to maintain a constant $^{15}N_2$ %atom throughout the incubation (Mohr et al., 2010), similar to the method described in Glibert and Bronk (1994). However, the $^{15}N_2$ dissolution method may introduce contaminants such as nutrients or trace metals, which can alter the diazotrophic activities and impact the accuracy of N_2 fixation measurements (Klawonn et al., 2015). Additionally, the pH and other chemical properties of the inoculum may be altered during its preparation, further affecting the measurement of N_2 fixation. Despite these limitations, the $^{15}N_2$ dissolution method remains the predominant assay for measuring N_2 fixation rate due to its ability to satisfy the fundamental assumption of constant $^{15}N_2$ %atom over the incubation.

More recently, a modified bubble method, known as the "bubble release method', has been proposed as an alternative to the $^{5}N_2$ dissolution method (Klawonn et al., 2015; Chang et al., 2019; White et al., 2020). This method involves adding $^{15}N_2$ gas to the incubation botttles and mixing for less than 15 minutes to facilitate $^{15}N_2$ equilibration, followed by releasing the gas bubbles and replacing them with $^{15}N_2$-unenriched seawater samples. Compared to the original bubble method, the bubble release method ensures a uniform $^{15}N_2$ %atom throughout the incubation. Moreover, it causes less invasion for the incubation matrix than the $^{15}N_2$ dissolution method and causes less interference with the incubation matrix. However, the agitation of incubation bottles required to stimulate gas dissolution may affect diazotrophs, such as *Trichodesumium* colonies (Wannicke et al., 2018; White et al., 2020). Moreover, the bubble release method results in increased spatial and labor expenditures, thereby impeding its widespread implementation (White et al., 2020). "

The first sentence of 4.1has also been revised as:

"To date, the discrepancy in N_2 fixation rates estimated using the original $^{15}N_2$ bubble method, the $^{15}N_2$ dissolution method, and the $^{15}N_2$ bubble release method remains unclear."

L150: There are 4 UCYN-A sublineages (Farnelid et al. 2016).

Response: We have corrected the text as follows:

"Four sublineages of UCYN-A, including UCYN-A1, UCYN-A2, UCYN-A3, and UCYN-A4, have been identified, with the clade UCYN-A1 sharing the same genome as previously targeted UCYN-A (Thompson et al., 2014; Farnelid et al., 2016)."

L328: UCYN-A has been found in symbiosis with other eukaryotic algae (Zehr et al. 2016)

Response: The text has been revised as:

"The conversion factor for UCYN-A is also updated because it has been found to live

symbiotically with prymnesiophytes, coccolithophores or other uncultured eukaryotic algae (Zehr et al., 2016; Thompson et al., 2012)."

L370: The first version of the database included all the authors that had contributed to its construction with their seagoing expeditions, laboratory analyses and publications. I humbly find it sad and somewhat unfair that this is not the case in this update.

Response: We highly value the reviewer's comment and agree proper coauthorship credit is important for all contributors. Initially, our plan to publish an updated global marine diazotrophic database was too simplistic and lacked careful thinking. Since the first global marine diazotrophic database was published in 2012, our group has continuously updated the database with newly published data. In recent years, we have received numerous requests for an updated version of the database, which prompted us to consider publishing it for wider usage.

We have extended an invitation to all PIs to join us as coauthors of the manuscript. Approximately 80 PIs have accepted and have become coauthors of the revised manuscript. We believe that this effort not only provides proper credit all involved contributors but also improves the quality and completeness of the database and accompanying paper.

Reviewer #2:

This manuscript by Shao and Xu et al. describes an updated version 2 of the global oceanic diazotroph database. It build upon the previous version by adding additional measurements of marine diazotrophic abundance, N_2 fixation rates, microscopic and qPCR-based diazotropic abundance. The spatial coverage significantly improved most notably in the Indian Ocean. The newly revised estimate for global N_2 fixation rate is significantly higher (+123 Tg N yr^{-1}, almost doubled) when calculating using a standard arithmetic mean, although surprisingly the geometric mean did not significantly change. A brief analysis and discussion of the $^{15}N_2$ bubble vs. dissolution indicated a potential general underestimation from the bubble method particularly at high rates, however noting the comparison of samples were from different times so it is not a formal error analysis (which the authors acknowledge). The database is available to download from the provided link in the abstract.

Overall, I find this to be an important update to the database mainly due to the significant increase in included measurements and spatial coverage. The database is transparent and mostly well described. The analysis and first preliminary quantification of the $^{15}N_2$ bubble vs. dissolution is also an important contribution. Perhaps some additional details/analysis could be provided (see comments below), but additional analyses can also be performed independently by users who download the data for their specific interest. There is one important aspect that needs additional clarification in my view before I would endorse this manuscript for publication (global N_2 fixation rate calculation, see below).

-Christopher Somes
GEOMAR Helmholtz Centre for Ocean Research Kiel

Response: We thank Dr. Somes for his positive and constructive comments, which have helped us improve the quality of this paper substantially. Please see our responses below.

Major Comment: Global N2 fixation calculation description

Since this paper will likely often be cited for revising the global N_2 fixation rate significantly upwards, the description of this calculation should be more transparent and comprehensive:

line 266 (Table 5 caption): “Data are first binned to 3x3 grids…”

This needs to be better described. For example, was there any type of interpolation method used or simple averaging of all measurements in each bin? It would be interesting to know what percentage of bins in each ocean basin has data coverage. How do you define the Southern Ocean region and is that area removed from the other southern regions?

How was the vertical coordinate handled? Is it evenly spaced or according to the depths ranges in Figure 7?

It is not clear to me how the “Areal sum” calculation was made based on the “Mean N_2 fixation rate” (Table 5). Does the “Mean N_2 fixation” rate include all measurements or only the “Depth-integrated N_2 rates”, which requires 3 measurements in the vertical? If the vertical coordinate is uneven, do measurements that get binned into a larger volume in larger deeper layers have more weight on the depth-integrated rate than shallower layers?

When calculating the “Areal Sum”, do you assume that the “Mean N2 fixation rate” extrapolates across the entire region or do you only consider the area of the bins that have data coverage? For example, the Indian Ocean has about 36% of the bins compared to the South Pacific. Therefore I was expecting a much larger decrease when calculating the Areal Sum relative to the Mean N_2 fixation rate for the Indian Ocean compared to the South Pacific. However this relative decrease is quite subtle in Table 5 between these regions. I acknowledge there is no truly perfect way to estimate a global ocean N_2 fixation rate with the current coverage, but all of the assumptions and details that go into the calculation should be specifically stated and described.

Response: Here we respond Dr. Somes's general comments regarding the description of calculating the global marine N_2 fixation rate.

We followed the procedure used in the previous database paper (Luo et al., 2012) to estimate the global marine N_2 fixation rate. However, as reminded by the reviewer, we should describe the method in this paper, which has been added in section 2.2 in the revised manuscript. Please be aware that we have also decided that only the arithmetic means should be used and have removed the geometric means in estimating of the global marine N_2 fixation rate (see our response below).

Here are some quick answers to the reviewer's questions:

The data used in the estimation is the depth-integrated N_2 fixation rates integrated from surface to the depth of the deepest data (up to 200 m; see section 2.1). The measurements in each vertical profile were linearly interpolated, which was not clearly described in the original manuscript. We have revised the sentence (in Section 2.1) to: "A profile was integrated from sea surface down to the deepest datum measured. The measurements within the profile were interpolated linearly along the depth, with the shallowest datum representing the level between the sea surface and that datum."

The arithmetic mean of the data in each bin was calculated first, and then these means in each basin were averaged further.

The Southern Ocean was defined as the area south of 45°S and was excluded from other basins when calculating the global rates. Additionally, due to very limited data coverage, the N_2 fixation rates of the Southern and Arctic Oceans have been excluded from the estimation of global marine N_2 fixation.

The percentage of bins with data coverage in each ocean basin have been added in the revised table.

When calculating the areal sum, we extrapolated the mean N_2 fixation rate of each basin across the entire basin, i.e., the mean N_2 fixation rate was multiplied by the area of each basin. We have listed the areas of every ocean basin in the table.

The description of the methods in calculating global marine N_2 fixation rate was added to Section 2.2:

"A first-order estimate of global marine N_2 fixation rate was conducted using data from this database. Total N_2 fixation rates were estimated for ocean basins including the North Atlantic, South Atlantic, North Pacific, South Pacific, Indian, Arctic, Southern Oceans, and the Mediterranean Sea. The Southern Ocean was defined as the region between 45°S and Antarctica. Due to considerable uncertainties associated with the acetylene reduction method, only N_2 fixation rates measured using the $^{15}N_2$ assimilation methods were used in this estimation. To increase data coverage, N_2 fixation rates measured using the original $^{15}N_2$ bubble method were included in the estimation, although it is acknowledged that these data may underestimate of the global marine N_2 fixation rate. First, the arithmetic means of depth-integrated N_2 fixation rates in each 3°×3° bin were calculated. Second, these binned means were further averaged in each ocean basin to obtain the average N_2 fixation rate, which was then multiplied by the basin area to estimate the total N_2 fixation rate for that basin. Finally, the global marine N_2 fixation rate was calculated by summing the basin rates, except for those of the Southern and Arctic Oceans due to limited spatial coverage in these two basins."

The authors do not give much context on interpreting the geometric vs. arithmetic mean despite that it is mentioned multiple times throughout the manuscript and gives a significantly different result. From what I understand, geometric mean is less sensitive to the high-end rates compared to arithmetic mean. Does this mean that most of the increase in the arithmetic mean is driven by newly included high-end rates? It would be valuable to know how much of the large increase in the arithmetic areal sum is driven by additional spatial coverage versus generally higher rate values. I would suggest to include a histogram of the previous version in one of the supplementary figures for comparison. If newly included rate values tend to be significantly higher, it would be interesting to know how much of that may be attributable to growing numbers of the dissolution method compared to bubble method (i.e. based on Figure 10).

Response: Dr. Somes was correct in interpreting geometric versus arithmetic means. As our N_2 fixation data were approximately log-normally distributed, their geometric mean is near the most frequently observed rate (i.e., the peaks of the distribution of the log-transformed N_2 fixation rates). Meanwhile, high N_2 fixation rates do occur and should be included in estimating

global N_2 fixation. Hence, the arithmetic means should be used in estimating global N_2 fixation if sufficient data have been sampled. However, if the number of samples is small, some occasionally observed high N_2 fixation rates can greatly elevate the estimated global rate while we cannot know if these high N_2 fixation rates are typical. This was the reason that we c presented both the geometric and arithmetic means of N_2 fixation rate in our 2012 paper and in the initial submission of the current manuscript.

With much more measurements becoming available, we have decided to only calculate arithmetic means of N_2 fixation in the revised manuscript. By this way, it can avoid the confusion of some readers in choosing proper estimations of total N_2 fixation rate of ocean basins and the global ocean. Additionally, N_2 fixation rates in most basins approximately follow log-normal distributions (except several data in the Indian Ocean, see below), indicating that most high N_2 fixation rates are acceptable.

In the initial version of this manuscript, the increase in the arithmetic-mean-based estimation of global marine N_2 fixation, compared to that in Luo et al. (2012), was caused mostly by (1) the nearly doubled rate in the South Pacific Ocean and (2) the high rate in the Indian Ocean for which the estimation of N_2 fixation was not made in Luo et al. (2012).

The much higher estimation of N_2 fixation rate in the South Pacific Ocean mostly attributes to the high rates sampled in the western South Pacific (new Fig. S9 attached below) where N_2 fixation rates were under sampled in the 2012 database. Overall, the N_2 fixation rate data in the South Pacific Ocean in the new database were close to log-normal distribution (new Fig. 10a attached below) and we used all of them to estimate the basin-wide rate.

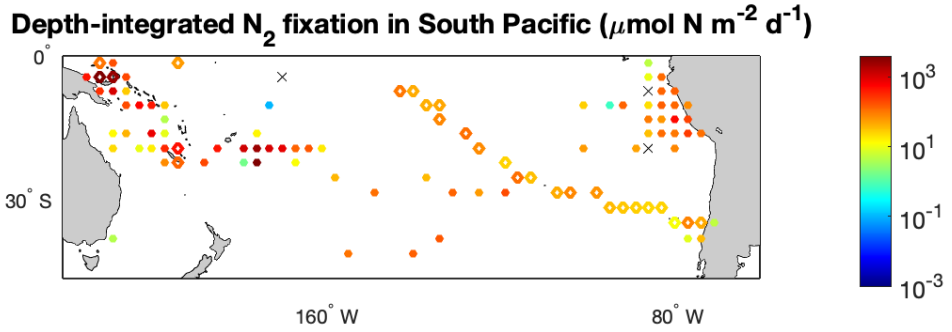

Figure S9. Depth-integrated N_2 fixation rates in the South Pacific Ocean. Filled circles represent the added data in the new database. Empty diamonds represent the data existing in the original database.

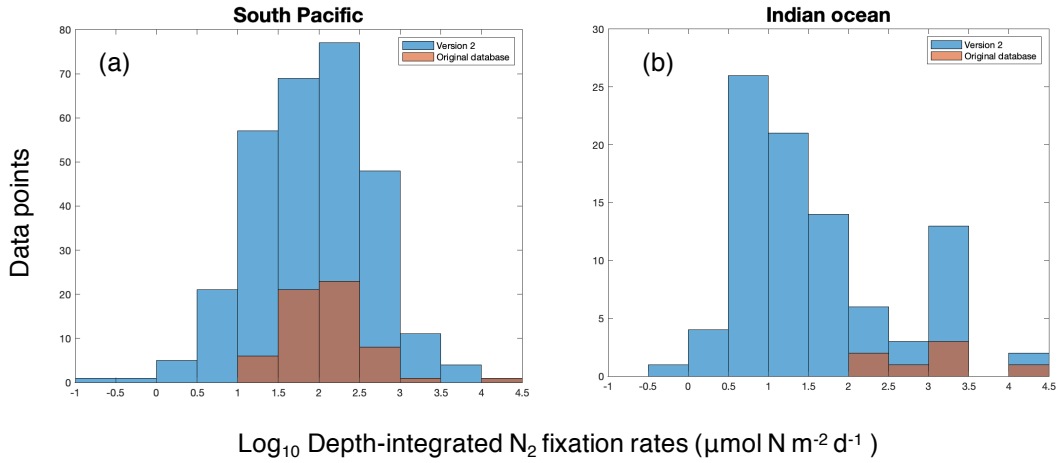

Figure 10. Histogram of depth-integrated N_2 fixation rates in (a) South Pacific and (b) Indian ocean.

The high estimation of the N_2 fixation rate of the Indian Ocean, however, was mainly caused by 14 extremely high measurements of N_2 fixation (> 1000 µmol N m^{-2} d^{-1}) sampled near the coast of western India. These high rates are much higher than all the other measurements in the Indian Ocean (Fig. 10b attached above) and are unlikely typical in the Indian Ocean. We have decided to remove them in our revised estimation, which greatly reducing the estimation of the N_2 fixation of the Indian Ocean from 98 Tg N yr^{-1} to 16 Tg N yr^{-1}.

Minor Comments:

line 84 and data file: Metadata
In the data file, the meta data are titled "Surface …", yet they are associated with a specific depth, so are they really surface? I am used to seeing chlorophyll expressed by volume not area.

Response: Thank you for pointing out the mistakes. In the volumetric spreadsheets, the meta data were measured at the same depths as the diazotrophic data, and the word "surface" has been deleted from their names. Similarly, the chlorophyll concentration in the volumetric datasheets should be in unit of mg m^{-3}, which has been corrected in the revised database.

In the depth-integrated datasheet, considering the large vertical variations of environmental parameters and chlorophyll, we collected their near-surface values. We have corrected their names to "Near-surface xx".

lines 127-129: daily vs. daytime vs. nighttime normalization
I am still a little confused about the time normalization with this brief description. If the incubation is only performed during the day, you convert hours to day by 12 hr/day which assumes no rates at night? I see that incubation hours vary a lot and in some cases not a multiple of 12 hours or 1 day. Perhaps you can describe how individual studies typically convert to a daily rate depending on the incubation period. Would it make more sense to multiply by the daytime of each location during the time of sampling instead of assuming 12 hours?

Response: We have changed the way to convert hourly N_2 fixation rates to daily rates according to the suggestions of Dr. Somes. The method has now been descried with more details:

"The majority of N_2 fixation rates (6766) were reported on a daily basis, while 1097 samples reported hourly N_2 fixation rates. We also converted these hourly rates to daily rates. In each sample, hourly N_2 fixation rates for daytime and nighttime were first multiplied by the respective local durations of day and night, and were then added to obtain the daily rate. However, 777 samples had hourly N_2 fixation measured only during daytime, which could have led to an underestimation of daily N_2 fixation because nighttime N_2 fixation was not included. It is important to note that diel cycles of N_2 fixation vary among samples and/or diazotrophic groups, and thus, substantial errors may be introduced when extrapolating these hourly N_2 fixation to daily rates (White et al., 2020)."

Table 5.: "n" is missing in Indian Ocean

Response: Corrected.

Figure 7:
Why do you choose geometric mean over the more commonly used arithmetic mean in this figure? Does it look significantly different if you use arithmetic means?

Response: The general spatial pattern of N_2 fixation was similar when using either geometric or arithmetic means, except for some high arithmetic means. Nevertheless, we have discarded the usage of geometric means in global marine N_2 fixation rates (see our response above); we then have changed to present arithmetic means of N_2 fixation in the revised manuscript (Fig. 7 attached below).

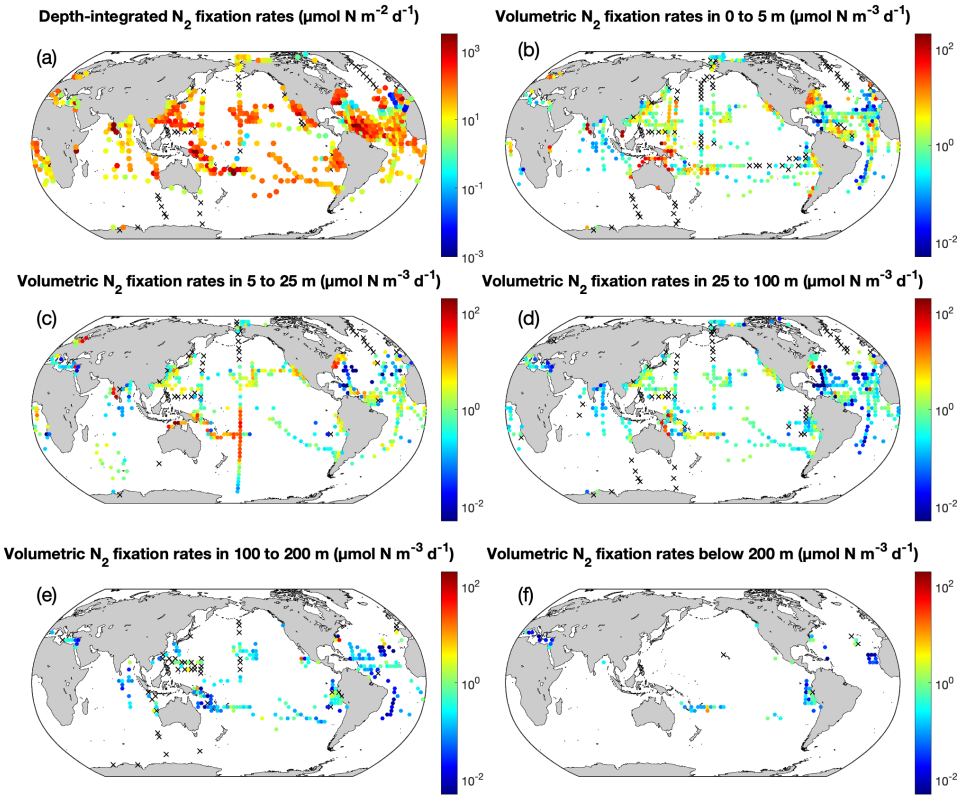

Figure 7. N_2 fixation rates in version 2 of the database. The panels show (**a**) depth-integrated

data and volumetric data in (**b**) 0–5 m, (**c**) 5–25 m, (**d**) 25–100 m, (**e**) 100-200 m, and (**f**) below 200 m. For a clear demonstration, data are binned to 3° × 3° grids and arithmetic means in each bin are shown. Zero-value data are denoted as black crosses.

I would be interested to see a euphotic vs. aphotic depth-integrated rate. I am curious how much the generally low to moderate rates occurring below 100 meters contribute to the total depth-integrated rate since they can occupy more volume. Perhaps adding a < 100m and >100m panel would be useful? At what depths are the deepest N2 fixation measurements?

Response: We have generated an averaged vertical profile of N_2 fixation rates from sea surface to the deepest (4000 m; Hallstrøm et al., 2022) N_2 fixation measured (Figure attached below). In the revised manuscript, we will present this figure and will compare and discuss the N_2 fixation rates above and below 200 m.

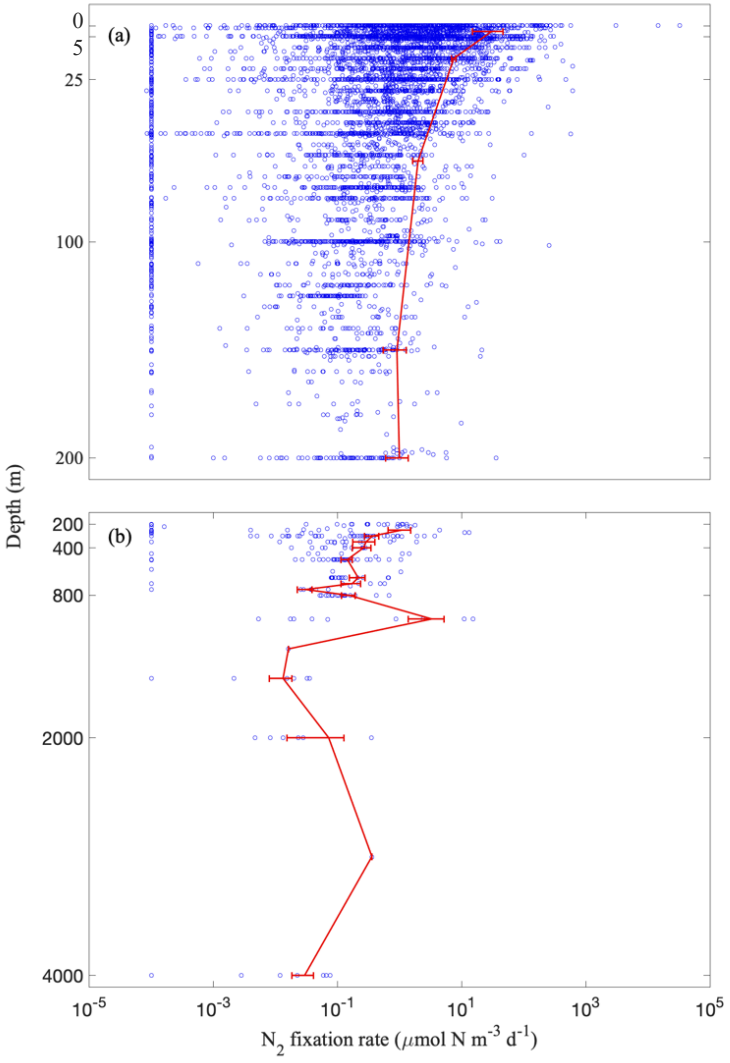

Figure. Vertical profile of N_2 fixation rates in the global ocean. Blue circles represent the reported N_2 fixation rates, and the red circles and error bars are the means and standard errors in depth intervals marked in the y-axis. The x-axis is in a log scale to better show the distribution of low N_2 fixation rates.

Section 4.1/Figure 10:
As mentioned above, I think is a useful first investigation into methodological uncertainties on N2 fixation rates. Is there enough data coverage to do a similar analysis for acetylene reduction?

Response: We thank Dr. Somes to recognize the value of our analyses. We compared the N_2 fixation rates measured using the $^{15}N_2$ tracer methods and from the acetylene reduction (ARA) method. However, there were too limited pairs of data available (n=28 and 24 for ARA vs. $^{15}N_2$ dissolution method and ARA vs. the original method, respectively) (see Figure S10 attached blow) to be included in the manuscript.

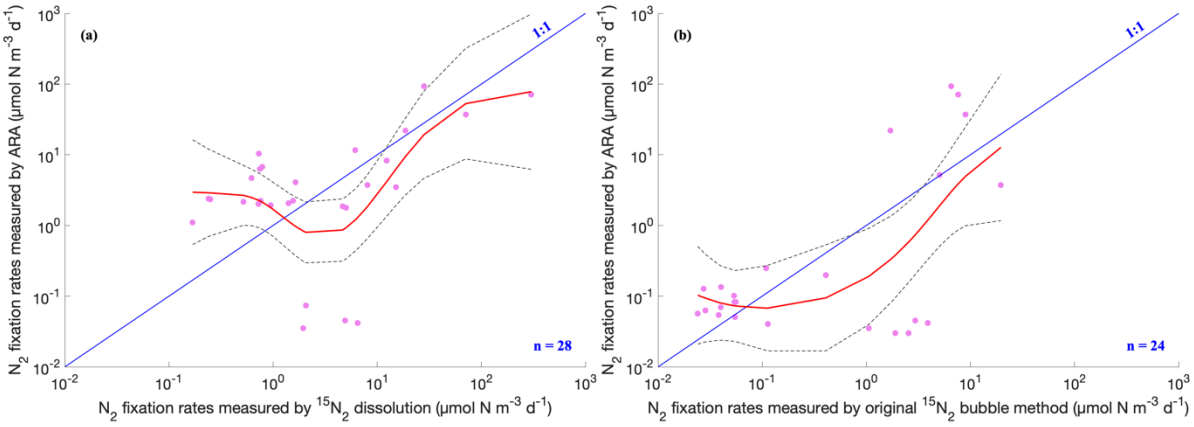

Figure S10. Comparison of measured N_2 fixation rates using the $^{15}N_2$ tracer methods and acetylene reduction (ARA) assays. (a) The ARA versus the $^{15}N_2$ dissolution; and (b) the ARA versus the original $^{15}N_2$ bubble method. The pink dots are measurements. The fitted results using the generalized additive model (GAM) and confidence intervals are represented by the red solid and the dashed black lines, respectively. The blue lines are the 1:1 ratio of the measurements using the compared methods.

Please be also noted that there were mistakes when pairing $^{15}N_2$ dissolution and $^{15}N_2$ bubbling measurements in the original manuscript, which have been corrected in the revised manuscript (new Fig. 11 attached below). The texts have been revised as follows:

"We also compared mean N_2 fixation rates at the same locations (using 3° × 3° grids), depth intervals (as defined in Fig. 7b–f) and months, using the original $^{15}N_2$ bubble method, the $^{15}N_2$ dissolution method, and the acetylene reduction assays, although the samples measured by these methods were not identical. The results showed that, in 68% of cases (**Fig. 11**), the $^{15}N_2$ dissolution method produced higher rates than the original $^{15}N_2$ bubble method. Furthermore, our analysis using the generalized additive model (GAM) indicated that the underestimation by the original $^{15}N_2$ bubble method tended to be exaggerated under high N_2 fixation (> ~5 μmol N m^{-3} d^{-1}) (**Fig. 11**). This can be explained by the gas equilibrium time (Jayakumar et al., 2017; Mohr et al., 2010). Under low N_2 fixation, the original $^{15}N_2$ bubble method can provide sufficient dissolved $^{15}N_2$ regardless of whether the gas reaches equilibrium. However, under

high N_2 fixation, the method cannot fulfill the requirement of dissolved $^{15}N_2$, resulting in relatively large underestimations.

We also used the same procedure to compare the N_2 fixation rates measured using ARA and the $^{15}N_2$ tracer methods. However, we had insufficient pairs of data available (n = 28 and 24 for ARA versus the $^{15}N_2$ dissolution or the original $^{15}N_2$ bubble method, respectively) for a robust comparison (Fig. S10)."

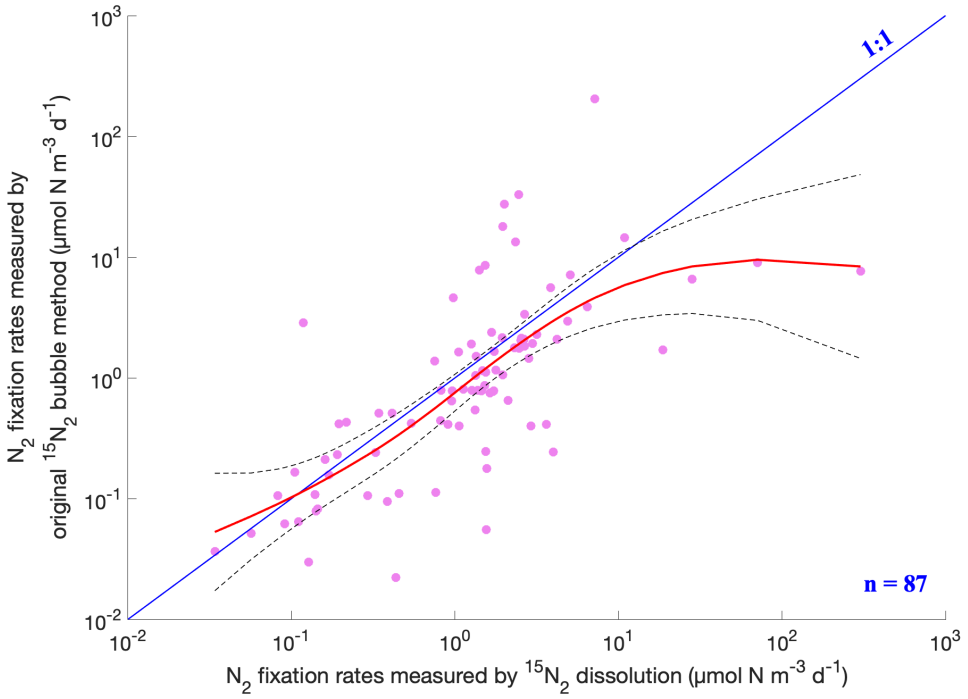

Figure 11. Comparison of measured N_2 fixation rates using the original $^{15}N_2$ bubble method and the $^{15}N_2$ dissolution method. The pink dots are measurements. The fitted results of the two methods by the generalized additive model (GAM) and confidence intervals are represented by the red solid line and the dashed black lines, respectively. The blue line represents the 1:1 ratio of the two methods.

References

Barthel K-G, S. G., Gradinger R, Lenz J: Concentration of live pico- and nanoplankton by means of tangential flow filtration, Journal of Plankton Research, 11, 1213-1221, https://doi.org/10.1093/plankt/11.6.1213, 1989.

Benavides, M., Santana-Falcón, Y., Wasmund, N., and Aristegui, J.: Microbial uptake and regeneration of inorganic nitrogen off the coastal Namibian upwelling system, Journal of Marine Systems, https://doi.org/10.1016/j.jmarsys.2014.05.002, 2014.

Benavides, M., Berthelot, H., Duhamel, S., Raimbault, P., and Bonnet, S.: Dissolved organic matter uptake by *Trichodesmium* in the Southwest Pacific, Scientific Reports, 7, 41315,

https://doi.org/10.1038/srep41315, 2017.

Benavides, M., Conradt, L., Bonnet, S., Berman-Frank, I., Barrillon, S., Petrenko, A., and Doglioli, A.: Fine-scale sampling unveils diazotroph patchiness in the South Pacific Ocean, ISME Communications, 1, 3, https://doi.org/10.1038/s43705-021-00006-2, 2021.

Bhavya, P. S., Min, J.-O., Kim, M.-S., Jang, H. K., Kim, K., Kang, J. J., Lee, J. H., Lee, D., Jo, N., Kim, M. J., Kim, Y., Lee, J., Lee, C. H., Bae, H., Yoo, H., Park, S., Yun, M. S., and Lee, S. H.: A review on marine N_2 fixation: mechanism, evolution of methodologies, rates, and future concerns, Ocean Science Journal, 54, 515-528, https://doi.org/10.1007/s12601-019-0037-3, 2019.

Bonnet, S., Dekaezemacker, J., Turk-Kubo, K. A., Moutin, T., Hamersley, R. M., Grosso, O., Zehr, J. P., and Capone, D. G.: Aphotic N_2 Fixation in the Eastern Tropical South Pacific Ocean, PLoS ONE, 8, e81265, https://doi.org/10.1371/journal.pone.0081265, 2013.

Bonnet, S., Benavides, M., Le Moigne, F. A. C., Camps, M., Torremocha, A., Grosso, O., Dimier, C., Spungin, D., Berman-Frank, I., Garczarek, L., and Cornejo-Castillo, F. M.: Diazotrophs are overlooked contributors to carbon and nitrogen export to the deep ocean, ISME J, 17, 47-58, https://doi.org/10.1038/s41396-022-01319-3, 2023.

Bonnet, S., Rodier, M., Turk-Kubo, K. A., Germineaud, C., Menkes, C., Ganachaud, A., Cravatte, S., Raimbault, P., Campbell, E., Quéroué, F., Sarthou, G., Desnues, A., Maes, C., and Eldin, G.: Contrasted geographical distribution of N_2 fixation rates and *nifH* phylotypes in the Coral and Solomon Seas (southwestern Pacific) during austral winter conditions, Global Biogeochemical Cycles, 29, 1874-1892, https://doi.org/10.1002/2015gb005117, 2015.

Capone, D. G.: Determination of nitrogenase activity in aquatic samples using the acetylene reduction procedure, in: Handbook of Methods in Aquatic Microbial Ecology, edited by: Kemp, P. F., Cole, J. J., Sherr, B. F., and Sherr, E. B., Lewis Publishers, Boca Raton, FL, 621–631, 1993.

Capone, D. G., Burns, J. A., Montoya, J. P., Subramaniam, A., Mahaffey, C., Gunderson, T., Michaels, A. F., and Carpenter, E. J.: Nitrogen fixation by *Trichodesmium* spp.: An important source of new nitrogen to the tropical and subtropical North Atlantic Ocean, Global Biogeochemical Cycles, 19, n/a-n/a, https://doi.org/10.1029/2004gb002331, 2005.

Cerdan-Garcia, E., Baylay, A., Polyviou, D., Woodward, E. M. S., Wrightson, L., Mahaffey, C., Lohan, M. C., Moore, C. M., Bibby, T. S., and Robidart, J. C.: Transcriptional responses of *Trichodesmium* to natural inverse gradients of Fe and P availability, The ISME Journal, 16, 1055-1064, https://doi.org/10.1038/s41396-021-01151-1, 2022.

Chang, B. X., Jayakumar, A., Widner, B., Bernhardt, P., Mordy, C. W., Mulholland, M. R., and Ward, B. B.: Low rates of dinitrogen fixation in the eastern tropical South Pacific, Limnol Oceanogr, 64, 1913-1923, https://doi.org/10.1002/lno.11159, 2019.

Church, M. J., Short, C. M., Jenkins, B. D., Karl, D. M., and Zehr, J. P.: Temporal Patterns of Nitrogenase Gene (*nifH*) Expression in the Oligotrophic North Pacific Ocean, Applied and Environmental Microbiology, 71, 5362-5370, https://doi.org/10.1128/aem.71.9.5362-5370.2005, 2005.

Cornejo-Castillo, F. M. and Zehr, J. P.: Intriguing size distribution of the uncultured and

globally widespread marine non-cyanobacterial diazotroph Gamma-A, ISME J, 5, https://doi.org/10.1038/s41396-020-00765-1, 2020.

Estrada, M., Delgado, M., Blasco, D., Latasa, M., Cabello, A. M., Benítez-Barrios, V., Fraile-Nuez, E., Mozetič, P., and Vidal, M.: Phytoplankton across Tropical and Subtropical Regions of the Atlantic, Indian and Pacific Oceans, https://doi.org/10.1371/journal.pone.0151699, 2016.

Farnelid, H., Turk-Kubo, K., Muñoz-Marín, M. C., and Zehr, J. P.: New insights into the ecology of the globally significant uncultured nitrogen-fixing symbiont UCYN-A, Aquat Microb Ecol, 77, 125-138, https://doi.org/10.3354/ame01794, 2016.

Flett, R. J., Hamilton, R. D., and Campbell, N. E. R.: Aquatic acetylene-reduction techniques: solutions to several problems, Canadian journal of microbiology, 22 1, 43-51, https://doi.org/10.1139/m76-006, 1976.

Foster, R. A., Tienken, D., Littmann, S., Whitehouse, M. J., Kuypers, M. M. M., and White, A. E.: The rate and fate of N2 and C fixation by marine diatom-diazotroph symbioses, ISME J, 16, 477-487, https://doi.org/10.1038/s41396-021-01086-7, 2022.

Geisler, E., Bogler, A., Bar-Zeev, E., and Rahav, E.: Heterotrophic nitrogen fixation at the hyper-eutrophic qshon river and estuary system, Front Microbiol, 11, 1370, https://doi.org/10.3389/fmicb.2020.01370, 2020.

Giller, K. E., Nambiar, P. T. C., Srinivasa Rao, B., Dart, P. J., and Day, J. M.: A comparison of nitrogen fixation in genotypes of 420 groundnut (Arachis hypogaea L.) using ^{15}N-isotope dilution, Biology and Fertility of Soils, https://doi.org/10.1007/BF00264341, 1987.

Glibert, P. M. and Bronk, D. A.: Release of Dissolved Organic Nitrogen by Marine Diazotrophic Cyanobacteria, *Trichodesmium* spp, Applied and environmental microbiology, 60, 3996-4000, https://doi.org/10.1128/aem.60.11.3996-4000.1994, 1994.

Gradoville, M. R., Dugenne, M., Hynes, A. M., Zehr, J. P., and White, A. E.: Empirical relationship between *nifH* gene abundance and diazotroph cell concentration in the North Pacific Subtropical Gyre, J Phycol, 58, 829-833, https://doi.org/10.1111/jpy.13289, 2022.

Gradoville, M. R., Bombar, D., Crump, B. C., Letelier, R. M., Zehr, J. P., and White, A. E.: Diversity and activity of nitrogen-fixing communities across ocean basins, Limnol Oceanogr, 62, 1895-1909, https://doi.org/10.1002/lno.10542, 2017.

Graham, J. A., Argyle, M., and Furnham, A.: The goal structure of situations, European Journal of Social Psychology, 10, 345-366, https://doi.org/10.1002/ejsp.2420100403, 1980.

Großkopf, T., Mohr, W., Baustian, T., Schunck, H., Gill, D., Kuypers, M. M. M., Lavik, G., Schmitz, R. A., Wallace, D. W. R., and LaRoche, J.: Doubling of marine dinitrogen-fixation rates based on direct measurements, Nature, 488, 361-364, https://doi.org/10.1038/nature11338, 2012.

Hallstrøm, S., Benavides, M., Salamon, E. R., Arístegui, J., and Riemann, L.: Activity and distribution of diazotrophic communities across the Cape Verde Frontal Zone in the Northeast Atlantic Ocean, Biogeochemistry, https://doi.org/10.1007/s10533-022-00940-w, 2022.

Harding, K., Turk-Kubo, K. A., Sipler, R. E., Mills, M. M., Bronk, D. A., and Zehr, J. P.: Symbiotic unicellular cyanobacteria fix nitrogen in the Arctic Ocean, P Natl Acad Sci USA, 115, 13371-13375, https://doi.org/10.1073/pnas.1813658115, 2018.

Harding, K. J., Turk-Kubo, K. A., Mak, E. W. K., Weber, P. K., Mayali, X., and Zehr, J. P.: Cell-specific measurements show nitrogen fixation by particle-attached putative non-cyanobacterial diazotrophs in the North Pacific Subtropical Gyre, Nat Commun, 13, 6979, https://doi.org/10.1038/s41467-022-34585-y, 2022.

Hardy, R. W. F., Burns, R. C., and Holsten, R. D.: Applications of the acetylene-ethylene assay for measurement of nitrogen fixation, Soil Biology and Biochemistry, 5, 47-81, https://doi.org/10.1016/0038-0717(73)90093-X, 1973.

Hyman, M. R. and Arp, D. J.: Quantification and removal of some contaminating gases from acetylene used to study gas-utilizing enzymes and microorganisms, Applied and environmental microbiology, 53, 298-303, https://doi.org/10.1128/aem.53.2.298-303.1987, 1987.

Jayakumar, A., Chang, B. X., Widner, B., Bernhardt, P., Mulholland, M. R., and Ward, B. B.: Biological nitrogen fixation in the oxygen-minimum region of the eastern tropical North Pacific ocean, The ISME Journal, 11, 2356-2367, https://doi.org/10.1038/ismej.2017.97, 2017.

Jiang, Z., Zhu, Y., Sun, Z., Zhai, H., Zhou, F., Yan, X., Zeng, J., Chen, J., and Chen, Q.: Enhancement of Summer Nitrogen Fixation by the Kuroshio Intrusion in the East China Sea and Southern Yellow Sea, Journal of Geophysical Research: Biogeosciences, 128, e2022JG007287, https://doi.org/10.1029/2022JG007287, 2023.

Kittu, L. R., Paul, A. J., Fernández-Méndez, M., Hopwood, M. J., and Riebesell, U.: Coastal N_2 Fixation Rates Coincide Spatially With Nitrogen Loss in the Humboldt Upwelling System off Peru, Global Biogeochemical Cycles, 37, https://doi.org/10.1029/2022gb007578, 2023.

Klawonn, I., Lavik, G., Boning, P., Marchant, H. K., Dekaezemacker, J., Mohr, W., and Ploug, H.: Simple approach for the preparation of $^{15\text{-}15}N_2$-enriched water for nitrogen fixation assessments: evaluation, application and recommendations, Front Microbiol, 6, 769, https://doi.org/10.3389/fmicb.2015.00769, 2015.

Landou, E., Lazar, B., LaRoche, J., Fennel, K., and Berman-Frank, I.: Contribution of photic and aphotic N_2 fixation to production in an oligotrophic sea, Limnology and Oceanography, 68, 692-708, https://doi.org/10.1002/lno.12303, 2023.

Luo, Y. W., Doney, S. C., Anderson, L. A., Benavides, M., Berman-Frank, I., Bode, A., Bonnet, S., Bostrom, K. H., Bottjer, D., Capone, D. G., Carpenter, E. J., Chen, Y. L., Church, M. J., Dore, J. E., Falcon, L. I., Fernandez, A., Foster, R. A., Furuya, K., Gomez, F., Gundersen, K., Hynes, A. M., Karl, D. M., Kitajima, S., Langlois, R. J., LaRoche, J., Letelier, R. M., Maranon, E., McGillicuddy, D. J., Moisander, P. H., Moore, C. M., Mourino-Carballido, B., Mulholland, M. R., Needoba, J. A., Orcutt, K. M., Poulton, A. J., Rahav, E., Raimbault, P., Rees, A. P., Riemann, L., Shiozaki, T., Subramaniam, A., Tyrrell, T., Turk-Kubo, K. A., Varela, M., Villareal, T. A., Webb, E. A., White, A. E., Wu, J., and Zehr, J. P.: Database of diazotrophs in global ocean: abundance, biomass and nitrogen fixation rates, Earth Syst. Sci. Data, 4, 47-73, https://doi.org/10.5194/essd-4-47-2012, 2012.

Mague, T. H., Weare, N. M., and Holm-Hansen, O.: Nitrogen fixation in the North Pacific Ocean, Marine Biology, 24, 109-119, https://doi.org/10.1007/bf00389344, 1974.

Messer, L. F., Brown, M. V., Van Ruth, P. D., Doubell, M., and Seymour, J. R.: Temperate southern Australian coastal waters are characterised by surprisingly high rates of nitrogen fixation and diversity of diazotrophs, PeerJ, 9, e10809, https://doi.org/10.7717/peerj.10809, 2021.

Mills, M. M., Turk-Kubo, K. A., van Dijken, G. L., Henke, B. A., Harding, K., Wilson, S. T., Arrigo, K. R., and Zehr, J. P.: Unusual marine cyanobacteria/haptophyte symbiosis relies on N2 fixation even in N-rich environments, ISME J, 14, 2395-2406, https://doi.org/10.1038/s41396-020-0691-6, 2020.

Mohr, W., Grosskopf, T., Wallace, D. W., and LaRoche, J.: Methodological underestimation of oceanic nitrogen fixation rates, PLoS One, 5, e12583, https://doi.org/10.1371/journal.pone.0012583, 2010.

Mompeán, C., Bode, A., Latasa, M., Fernández-Castro, B., and Irigoien, X.: Nitrogen inputs influence on biomass and trophic structure of ocean plankton : a study using biomass and stable isotope size-spectra, https://doi.org/10.1093/plankt/fbw052,

Montoya, J. P., Voss, M., Kahler, P., and Capone, D. G.: A Simple, High-Precision, High-Sensitivity Tracer Assay for N_2 Fixation, Appl Environ Microbiol, 62, 986-993, https://doi.org/10.1128/aem.62.3.986-993.1996, 1996.

Moore, R. M., Grefe, I., Zorz, J., Shan, S., Thompson, K., Ratten, J., and LaRoche, J.: On the relationship between hydrogen saturation in the tropical Atlantic Ocean and nitrogen fixation by the symbiotic diazotroph UCYN-A, J Geophys Res-Oceans, 123, 2353-2362, https://doi.org/10.1002/2017jc013047, 2018.

Moreira-Coello, V., Mourino-Carballido, B., Maranon, E., Fernandez-Carrera, A., Bode, A., and Varela, M. M.: Biological N_2 fixation in the upwelling region off NW Iberia: magnitude, relevance, and players, Front. Mar. Sci., 4, 303, https://doi.org/10.3389/fmars.2017.00303, 2017.

Mulholland, M. R.: The fate of nitrogen fixed by diazotrophs in the ocean, Biogeosciences, https://doi.org/10.5194/bg-4-37-2007, 2007.

Postgate, J. R.: The Fundamentals of Nitrogen Fixation, Cambridge University Press, New York1998.

Raes, E. J., Waite, A. M., McInnes, A. S., Olsen, H., Nguyen, H. M., Hardman-Mountford, N., and Thompson, P. A.: Changes in latitude and dominant diazotrophic community alter N_2 fixation, Marine Ecology Progress Series, 516, 85-102, https://doi.org/10.3354/meps11009, 2014.

Reeder, C. F., Stoltenberg, I., Javidpour, J., and Löscher, C. R.: Salinity as a key control on the diazotrophic community composition in the southern Baltic Sea, Ocean Sci., 18, 401-417, https://doi.org/10.5194/os-18-401-2022, 2022.

Sahu, B. K., Baliarsingh, S. K., Lotliker, A. A., Parida, C., Srichandan, S., and Sahu, K. C.: Winter thermal inversion and *Trichodesmium* dominance in north-western Bay of Bengal,

Ocean Science Journal, 52, 301-306, 10.1007/s12601-017-0028-1, 2017.

Sargent, E. C., Hitchcock, A., Johansson, S. A., Langlois, R., Moore, C. M., LaRoche, J., Poulton, A. J., and Bibby, T. S.: Evidence for polyploidy in the globally important diazotroph *Trichodesmium*, FEMS microbiology letters, 363, fnw244, https://doi.org/10.1093/femsle/fnw244, 2016.

Sato, T., Shiozaki, T., Taniuchi, Y., Kasai, H., and Takahashi, K.: Nitrogen fixation and diazotroph community in the subarctic Sea of Japan and Sea of Okhotsk, J Geophys Res-Oceans, 126, https://doi.org/10.1029/2020jc017071, 2021.

Sato, T., Shiozaki, T., Hashihama, F., Sato, M., Murata, A., Sasaoka, K., Umeda, S.-i., and Takahashi, K.: Low Nitrogen Fixation Related to Shallow Nitracline Across the Eastern Indian Ocean, Journal of Geophysical Research: Biogeosciences, 127, e2022JG007104, https://doi.org/10.1029/2022JG007104, 2022.

Saulia, E., Benavides, M., Henke, B., Turk-Kubo, K., Cooperguard, H., Grosso, O., Desnues, A., Rodier, M., Dupouy, C., Riemann, L., and Bonnet, S.: Seasonal Shifts in Diazotrophs Players: Patterns Observed Over a Two-Year Time Series in the New Caledonian Lagoon (Western Tropical South Pacific Ocean), Frontiers in Marine Science, 7, https://doi.org/10.3389/fmars.2020.581755, 2020.

Selden, C. R., Chappell, P. D., Clayton, S., Macías-Tapia, A., Bernhardt, P. W., and Mulholland, M. R.: A coastal N_2 fixation hotspot at the Cape Hatteras front: Elucidating spatial heterogeneity in diazotroph activity via supervised machine learning, Limnology and Oceanography, 66, 1832-1849, https://doi.org/10.1002/lno.11727, 2021.

Selden, C. R., Einarsson, S. V., Lowry, K. E., Crider, K. E., Pickart, R. S., Lin, P., Ashjian, C. J., and Chappell, P. D.: Coastal upwelling enhances abundance of a symbiotic diazotroph (UCYN-A) and its haptophyte host in the Arctic Ocean, Frontiers in Marine Science, 9, https://doi.org/10.3389/fmars.2022.877562, 2022.

Shao, Z. and Luo, Y.-W.: Controlling factors on the global distribution of a representative marine non-cyanobacterial diazotroph phylotype (Gamma A), Biogeosciences, 19, 2939-2952, https://doi.org/10.5194/bg-19-2939-2022, 2022.

Shiozaki, T., Ijichi, M., Kodama, T., Takeda, S., and Furuya, K.: Heterotrophic bacteria as major nitrogen fixers in the euphotic zone of the Indian Ocean, Global Biogeochemical Cycles, 28, 1096-1110, https://doi.org/10.1002/2014gb004886, 2014.

Shiozaki, T., Fujiwara, A., Inomura, K., Hirose, Y., Hashihama, F., and Harada, N.: Biological nitrogen fixation detected under Antarctic sea ice, Nat Geosci, 13, 729-+, https://doi.org/10.1038/s41561-020-00651-7, 2020.

Shiozaki, T., Bombar, D., Riemann, L., Sato, M., Hashihama, F., Kodama, T., Tanita, I., Takeda, S., Saito, H., Hamasaki, K., and Furuya, K.: Linkage between dinitrogen fixation and primary production in the oligotrophic South Pacific Ocean, Global Biogeochemical Cycles, 32, 1028-1044, https://doi.org/10.1029/2017gb005869, 2018.

Singh, A., Gandhi, N., and Ramesh, R.: Surplus supply of bioavailable nitrogen through N_2 fixation to primary producers in the eastern Arabian Sea during autumn, Continental Shelf Research, 181, 103-110, https://doi.org/10.1016/j.csr.2019.05.012, 2019.

Staal, M., te Lintel Hekkert, S., Jan Brummer, G., Veldhuis, M., Sikkens, C., Persijn, S., and Stal, L. J.: Nitrogen fixation along a north-south transect in the eastern Atlantic Ocean, Limnology and Oceanography, 52, 1305-1316, https://doi.org/10.4319/lo.2007.52.4.1305, 2007.

Tang, W., Cerdán-García, E., Berthelot, H., Polyviou, D., Wang, S., Baylay, A., Whitby, H., Planquette, H., Mowlem, M., Robidart, J., and Cassar, N.: New insights into the distributions of nitrogen fixation and diazotrophs revealed by high-resolution sensing and sampling methods, The ISME Journal, 14, 2514-2526, https://doi.org/10.1038/s41396-020-0703-6, 2020.

Tang, W. Y. and Cassar, N.: Data-driven modeling of the distribution of diazotrophs in the global ocean, Geophys. Res. Lett., 46, 12258-12269, https://doi.org/10.1029/2019gl084376, 2019.

Tang, W. Y., Wang, S., Fonseca-Batista, D., Dehairs, F., Gifford, S., Gonzalez, A. G., Gallinari, M., Planquette, H., Sarthou, G., and Cassar, N.: Revisiting the distribution of oceanic N_2 fixation and estimating diazotrophic contribution to marine production, Nat. Commun., 10, https://doi.org/10.1038/s41467-019-08640-0, 2019.

Tenorio, M. M. B., Dupouy, C., Rodier, M., and Neveux, J.: *Trichodesmium* and other planktonic cyanobacteria in New Caledonian waters (SW tropical Pacific) during an El Nino episode, Aquatic Microbial Ecology, 81, 219-241, https://doi.org/10.3354/ame01873, 2018.

Thompson, A., Carter, B. J., Turk-Kubo, K., Malfatti, F., Azam, F., and Zehr, J. P.: Genetic diversity of the unicellular nitrogen-fixing cyanobacteria UCYN-A and its prymnesiophyte host, Environ. Microbiol., 16, 3238-3249, https://doi.org/10.1111/1462-2920.12490, 2014.

Thompson, A. W., Foster, R. A., Krupke, A., Carter, B. J., Musat, N., Vaulot, D., Kuypers, M. M. M., and Zehr, J. P.: Unicellular cyanobacterium symbiotic with a single-celled eukaryotic alga, Science (New York, N.Y.), 337, 1546-1550, https://doi.org/10.1126/science.1222700, 2012.

Turk-Kubo, K., Gradoville, M., Cheung, S., Cornejo Castillo, F. M., Harding, K., Morando, M., Mills, M., and Zehr, J.: Non-cyanobacterial diazotrophs: Global diversity, distribution, ecophysiology, and activity in marine waters, FEMS microbiology reviews, https://doi.org/10.1093/femsre/fuac046, 2022.

Turk-Kubo, K. A., Mills, M. M., Arrigo, K. R., van Dijken, G., Henke, B. A., Stewart, B., Wilson, S. T., and Zehr, J. P.: UCYN-A/haptophyte symbioses dominate N_2 fixation in the Southern California Current System, ISME Communications, 1, 42, https://doi.org/10.1038/s43705-021-00039-7, 2021.

Wannicke, N., Benavides, M., Dalsgaard, T., Dippner, J. W., Montoya, J. P., and Voss, M.: New perspectives on nitrogen Fixation measurements using $^{15}N_2$ Gas, Frontiers in Marine Science, 5, https://doi.org/10.3389/fmars.2018.00120, 2018.

Wen, Z., Browning, T. J., Cai, Y., Dai, R., Zhang, R., Du, C., Jiang, R., Lin, W., Liu, X., Cao, Z., Hong, H., Dai, M., and Shi, D.: Nutrient regulation of biological nitrogen fixation across the tropical western North Pacific, Science advances, 8, eabl7564-eabl7564, https://doi.org/10.1126/sciadv.abl7564, 2022.

White, A. E., Watkins-Brandt, K. S., and Church, M. J.: Temporal variability of *Trichodesmium* spp. and diatom-diazotroph assemblages in the North Pacific subtropical gyre, Front. Mar. Sci., 5, 27, https://doi.org/10.3389/fmars.2018.00027, 2018.

White, A. E., Granger, J., Selden, C., Gradoville, M. R., Potts, L., Bourbonnais, A., Fulweiler, R. W., Knapp, A. N., Mohr, W., Moisander, P. H., Tobias, C. R., Caffin, M., Wilson, S. T., Benavides, M., Bonnet, S., Mulholland, M. R., and Chang, B. N. X.: A critical review of the N^{15}_2 tracer method to measure diazotrophic production in pelagic ecosystems, Limnology and Oceanography-Methods, 18, 129-147, https://doi.org/10.1002/lom3.10353, 2020.

Wilson, S. T., Böttjer, D., Church, M. J., and Karl, D. M.: Comparative Assessment of Nitrogen Fixation Methodologies, Conducted in the Oligotrophic North Pacific Ocean, Applied and Environmental Microbiology, 78, 6516-6523, https://doi.org/10.1128/aem.01146-12, 2012.

Zehr, J. P., Shilova, I. N., Farnelid, H. M., Munoz-Marin, M. D., and Turk-Kubo, K. A.: Unusual marine unicellular symbiosis with the nitrogen-fixing cyanobacterium UCYN-A, Nat Microbiol, 2, 16214, https://doi.org/10.1038/nmicrobiol.2016.214, 2016.