Version 2 of the global oceanic diazotroph database

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Abstract. Marine diazotrophs convert dinitrogen (N_2) in seawater into bioavailable nitrogen (N), contributing approximately half of the external input of bioavailable N to the global ocean. A global oceanic diazotroph database was previously published in 2012 (Luo et al., 2012). Here, we compiled version 2 of the database by adding 23,095 *in situ* measurements of marine diazotrophic abundance and N_2 fixation rates published in the past decade, increasing the number of N_2 fixation rates and microscopic and qPCR-based diazotrophic abundance data by 140%, 26% and 443%, respectively. Although the updated database expanded spatial coverage considerably, particularly in the Indian Ocean, the data distribution was still not uniform and most data were sampled in the surface Pacific and Atlantic Oceans. By summing the arithmetic means of the N_2 fixation rates in each ocean basin, the updated database substantially increased the estimate of global oceanic N_2 fixation from 137 ± 9 Tg N yr^{-1} using the old database to 260 ± 20 Tg N yr^{-1} (mean $\pm$ standard error). However, using geometric means instead, the updated database gave an estimate of global oceanic N_2 fixation (60 Tg N yr^{-1}) similar to that estimated from the old database (62 Tg N yr^{-1}), while the new estimate had a larger uncertainty (confidence intervals based on one standard error: 47 – 107 Tg N yr^{-1} versus 52 – 73 Tg N yr^{-1}), mostly attributable to elevated uncertainties in the Pacific Ocean. An analysis comparing N_2 fixation rates measured at the same months and location (1° × 1° grids) showed that the new $^{15}N_2$ dissolution method obtained N_2 fixation rates higher than the conventional $^{15}N_2$ bubble method in 65% of cases, with this percentage increasing when the N_2 fixation rates were high (> approximately 3 μmol N m^{-3} d^{-1} using the $^{15}N_2$ dissolution method). With greatly increased data points, this version 2 of the global oceanic diazotrophic database can support future studies in marine ecology and biogeochemistry. The database is stored at the Figshare repository (https://doi.org/10.6084/m9.figshare.21677687) (Shao et al., 2022).

1 Introduction

Dinitrogen (N_2) fixation is conducted by a group of microbes, termed diazotrophs, to convert inert N_2 gases into bioavailable nitrogen (N). In the ocean, where N nutrients are largely scarce, N_2 fixation plays an important role in fertilizing primary

30   production, particularly in oligotrophic regions (Gruber et al., 2008; Wang et al., 2019), and balances N loss processes such as denitrification and anammox (Deutsch et al., 2007; Gruber, 2019).

Marine diazotrophs include three main types of autotrophic cyanobacteria (Zehr, 2011): (1) nonheterocystous filamentous cyanobacteria, *Trichodesmium*; (2) heterocystous cyanobacteria, *Richelia* or *Calothrix*, forming diazotroph-diatom associations (DDAs); and (3) unicellular cyanobacteria (UCYNs).

35   Diazotrophic abundance can be directly obtained by counting their cells using microscopes or be estimated from their copies of *nifH* using qPCR assays (Church et al., 2005). *NifH* copies have been more frequently measured than microscopic cell counting, particularly in the past decade, and can be more useful when evaluating the abundance of different diazotrophic groups. However, caution must be taken because there are discrepancies between cell-count-based and *nifH*-based diazotrophic abundance (Luo et al., 2012), largely attributed to large variations in the number of *nifH* copies in the genomes of marine

40   diazotrophs, particularly *Trichodesmium* and heterocystous cyanobacteria (Sargent et al., 2016; White et al., 2018).

Considering the key role of N_2 fixation in oceans, a database compiling up-to-date measurements of N_2 fixation and diazotrophic abundances is essential in studying marine ecology and biogeochemistry. For example, the estimated global marine N_2 fixation rate ranges from 15 to 238 Tg N yr^{-1} using different methods (Zehr and Capone, 2021), which is commonly thought to be much lower than the estimated nitrogen loss (126 – 481 Tg N yr^{-1}) from denitrification and anammox (Zehr and

45   Capone, 2020; Gruber et al., 2008; Zhang et al., 2020). One possible reason 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).

Luo et al. (2012) compiled the first global oceanic diazotrophic database including *in situ* measurements of N_2 fixation rates and cell-count-based and *nifH*-based diazotrophic abundance. In the past decade, many more measurements have been

50   reported, extending diazotrophic activities to new habitats such as subpolar (Sato et al., 2021; Shiozaki et al., 2018b) and even polar regions (Shiozaki et al., 2020; Harding et al., 2018). Several years ago, two studies supplemented the database with some newly measured diazotrophic data (Tang and Cassar, 2019; Tang et al., 2019), although a substantial amount of additional data still needed to be included.

Here, we present an updated version of the global oceanic diazotrophic database. We describe the database information,

55   a summary of the data updates, measurement methods and data distribution. Furthermore, we conduct a first-order estimation of the global oceanic N_2 fixation rate using the updated database. We also analyzed the discrepancy in N_2 fixation assays and the relationship between *nifH* gene copies and diazotrophic cell abundance based on the existing data.

2 Data and methods

2.1 Database summary

60   This study updated the original global oceanic diazotrophic database of Luo et al. (2012) with new *in situ* measurements of N_2 fixation rates and abundance of diazotrophic cells and *nifH* gene copies. The updated database (version 2) included 23,095 new data points, including 6,902 measured in 2012 – 2018 and collected by two previous studies (Tang et al., 2019; Tang and Cassar, 2019) and 16,193 data points measured in 2012 – 2022 and collected by this study (**Table 1**). In version 2 of the database, some errors in the datasets of Tang et al. (2019) (mostly caused in unit conversions) were also corrected. Version 2
65   of the database is composed of six sub-databases: (1) 7,515 volumetric N_2 fixation rates (4,200 new data points) (**Tables 1 & 2**); (2) 2,248 depth-integrated N_2 fixation rates (1,497 new data points) (**Tables 1 & 2**); (3) 6,016 volumetric cell counts (1,130 new data points) (**Tables 1 & 3**); (4) 1,291 depth-integrated cell counts (360 new data points) (**Tables 1 & 3**); (5) 17,143 volumetric *nifH* copies (13,994 new data points) (**Tables 1 & 4**); and (6) 2,356 depth-integrated *nifH* copies (1,914 new data points) (**Tables 1 & 4**).

70   More recently, a unique group of non-cyanobacterial diazotrophs (NCDs) carrying *nifH* (gene encoding N_2-fixing enzyme, nitrogenase) has been widely found (Moisander et al., 2017; Zehr et al., 1995; Zehr, 1998), although direct evidence of N_2 fixation by NCDs is still limited (Harding et al., 2022). We therefore did not include NCD data in our database, while those who would be interested can use two recently published NCD datasets (Shao and Luo, 2022; Turk-Kubo et al., 2022).

Depth-integrated data were either provided directly in published papers or calculated for those vertical profiles with at
75   least 3 volumetric data points in each profile. A profile was integrated from the sea surface to the depth of the deepest datum, while using the value of the shallowest datum to represent the level in the upper layer. We ignored those rates of N_2 fixation deeper than 200 m when calculating depth integrals, because they often had low vertical resolutions.

As in the original database, the 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,
80   and the heterocystous cyanobacterial abundance was grouped into *Richelia* and *Calothrix*. N_2 fixation rates were measured for size groups or in whole seawater samples. When whole-water N_2 fixation rates were not reported, total N_2 fixation rates were calculated as the sum of N_2 fixation rates of different groups.

Sampling information (latitude, longitude, depth and time) was provided for each data point. Physical, chemical and biological parameters, including temperature, salinity, and concentrations of nitrate, phosphate, iron and chlorophyll, were also
85   included when they were available.

Table 1. Summary of data points for N_2 fixation rates, cell-count-based abundance and *nifH*-based abundance.

	Original database	New data added in Version 2			Sum
		Tang et al., 2019	Tang & Cassar, 2019	This study	
Volumetric N₂ fixation rate					
Trichodesmium	689	145		83	917
UCYN	275	124			399
Heterocystous	205	30		83	318
Whole seawater	2,146	1,322		2,413	5,881
Total	3,315	1,621		2,579	7,515
Proportion	48.1%	21.6%		34.3%	
Depth-integrated N₂ fixation rate					
Trichodesmium	280	89		45	414
UCYN	46	18		1	65
Heterocystous	65	92		20	177
Whole seawater	360	544		702	1606
Total	751	743		768	2262
Proportion	33.2%	32.8%		34.0%	
Volumetric cell-count-based data					
Trichodesmium	3,274			645	3,919
UCYN				85	85
Heterocystous	1,612			400	2,012
Total	4,886			1,130	6,016
Proportion	81.2%			18.8%	
Depth-integrated cell-count-based data					
Trichodesmium	626			241	867
UCYN				19	19
Heterocystous	305			100	405
Total	931			360	1,291
Proportion	72.1%			27.9%	
Volumetric *nifH*-based data					
Trichodesmium	758		770	2,382	3,910
UCYN	1,792		2,640	4,822	9,254
Heterocystous	599		505	2,875	3,979

Total	3,149	3915	10,079	17,143
Proportion	18.4%	22.8%	58.7%	
Depth-integrated *nifH*-based data				
Trichodesmium	105	123	297	525
UCYN	263	418	609	1,290
Heterocystous	74	82	385	541
Total	442	623	1,291	2,356
Proportion	18.8%	26.4%	54.8%	

Table 2. Summary of data points of N_2 fixation rates in version 2 of the database, including whole seawater and volumetric measurements for *Trichodesmium*, unicellular and heterocystous diazotrophs.

Reference	Region	*Tricho-desmium*	UCYN	Hetero-cystous	Whole Seawater	Depth-integrated
Agawin et al. (2013)	Subtropical Atlantic	21			17	
Ahmed et al. (2017)	E Arabian Sea				19	5[a]
Benavides et al. (2013)	Subtropical N Atlantic	15	15		15	
Benavides et al. (2016a)	Mediterranean Sea				10	
Benavides et al. (2018a)	Tropical SW Pacific				59	
Benavides et al. (2022)	S Pacific				38	
Bentzon-Tilia et al. (2015)	Baltic Sea				23	23[a]
Berthelot et al. (2017)	Tropical W Pacific				48	12[a]
Bhavya et al. (2016)	Arabian Sea				4	
Biegala and Raimbault (2008)	SW Pacific				9	2
Blais et al. (2012)	Arctic Ocean				18	12
Bombar et al. (2011)	South China Sea				15	
Bombar et al. (2015)	Subtropical N Pacific				20	2
Bonnet et al. (2013)	Tropical SW Pacific					8[a]
Bonnet et al. (2015)	SW Pacific					30[a]
Bonnet et al. (2018)	Tropical SW Pacific				102	14
Böttjer et al. (2017)	Subtropical N Pacific				243	108[a]
Chang et al. (2000)	S East China Sea					7[a]
Chang et al. (2019)	Tropical SE Pacific				37	
Dekaezemacker et al. (2013)	Tropical SE Pacific				43	10
Fernandez et al. (2015)	Central Chile Upwelling System				84	14[a]
Fernández-Castro et al. (2015)	Atlantic, Pacific and Indian Oceans					43[a]
Fonseca-Batista et al. (2017)	E Atlantic				56	14

Reference	Region	*Tricho-desmium*	UCYN	Hetero-cystous	Whole Seawater	Depth-integrated
Fonseca-Batista et al. (2019)	Temperate NE Atlantic				46	10[a]
Foster et al. (2009)	Red Sea				26	
Gandhi et al. (2011)	E Arabian Sea				28	7[a]
Garcia et al. (2007)	SW Pacific					1[a]
González et al. (2014)	Southern Ocean				8	
Gradoville et al. (2020)	N Pacific				20	
Großkopf et al. (2012)	Atlantic Ocean				39	17
Hallstrøm et al. (2022)	NE Atlantic				59	11[a]
Halm et al. (2012)	S Pacific	43	43		43	10[a]
Harding et al. (2018)	Arctic Ocean				38	
Hörstmann et al. (2021)	S Indian Ocean; Southern Ocean				13	
Ibello et al. (2010)	Mediterranean Sea				21	7[a]
Jayakumar et al. (2017)	Tropical NE Pacific				32	7[a]
Knapp et al. (2016)	Tropical SE Pacific					6[a]
Konno et al. (2010)	NW Pacific					16[a]
Kromkamp et al. (1997)	Indian Ocean					4[a]
Krupke et al. (2013)	Subtropical N Atlantic				3	
Krupke et al. (2014)	N Atlantic	42	42	30	42	44[a]
Krupke et al. (2015)	Subtropical NE Atlantic				1	
Kumar et al. (2017)	E Arabian Sea				12	3
Kumari et al. (2022)	Bay of Bengal				97	18[a]
Lee Chen et al. (2014)	South China Sea					24[a]
Li et al. (2020)	N South China Sea; East China Sea				68	15[a]
Liu et al. (2020)	South China Sea				25	5[a]
Loescher et al. (2014)	Pacific Ocean				30	5[a]
Loick-Wilde et al. (2015)	Amazon River					36[a]

Reference	Region	*Tricho-desmium*	UCYN	Hetero-cystous	Whole Seawater	Depth-integrated
Loick-Wilde et al. (2019)	Tropical W Pacific				8	
Lory et al. (2022)	Tropical SW Pacific				5	
Löscher et al. (2016)	Tropical SW Pacific				225	31[a]
Löscher et al. (2020)	Bay of Bengal				18	
Lu et al. (2018)	Equatorial W Pacific				3	3[a]
Martínez-Pérez et al. (2016)	Tropical N Atlantic				84	14
Messer et al. (2016)	S Pacific				27	
Mouriño-Carballido et al. (2011)	Atlantic Ocean					20[a]
Mulholland et al. (2019)	NW Atlantic				402	242[a]
Needoba et al. (2007)	Temperate N Pacific				2	1[a]
Raes et al. (2020)	S Pacific				55	
Rahav et al. (2013a); Rahav et al. (2015)	Red Sea and E Mediterranean Sea				62	10
Rahav et al. (2013b); Rahav et al. (2013c)	Mediterranean Sea				8	
Rahav et al. (2016)	Mediterranean Sea					3[a]
Reeder et al. (2022)	S Baltic Sea				15	5
Riou et al. (2016)	N Atlantic	24	24			6
Sahoo et al. (2021)	Bay of Bengal					6[a]
Sarma et al. (2020)	Bay of Bengal				2	
Sato et al. (2021)	Subarctic Sea of Japan; Sea of Okhotsk				31	3
Saxena et al. (2020)	Bay of Bengal				32	8
Selden et al. (2019)	Tropical NE Pacific				8	16[a]
Shiozaki et al. (2013)	W Pacific				50	10
Shiozaki et al. (2014a)	Indian Ocean				42	
Shiozaki et al. (2014b)	SW Pacific				26	6[a]
Shiozaki et al. (2015a)	NW Pacific				73	11[a]
Shiozaki et al. (2015b)	N Pacific				112	22[a]

Reference	Region	*Tricho-desmium*	UCYN	Hetero-cystous	Whole Seawater	Depth-integrated
Shiozaki et al. (2017)	N Pacific				74	15
Shiozaki et al. (2018b)	W Arctic Ocean				84	21[a]
Shiozaki et al. (2020)	Antarctic Coast				53	15[a]
Sipler et al. (2017)	Arctic Ocean				8	
Sohm et al. (2011)	S Atlantic				12	3[a]
Subramaniam et al. (2008)	Tropical N Atlantic					242[a]
Subramaniam et al. (2013)	Atlantic Ocean				96	24[a]
Tang et al. (2020)	N Atlantic				15	
Turk-Kubo et al. (2012)	Tropical N Atlantic				27	7
Wang et al. (2021)	NW Atlantic				85	
Wasmund et al. (2015)	S Atlantic					66[a]
Watkins-Brandt et al. (2011)	N Pacific					1[a]
Wen et al. (2022)	Tropical NW Pacific				143	22[a]
White et al. (2018)	Subtropical N Pacific	83		83	62	51[a]
Wilson et al. (2012)	N Pacific				9	4[a]
Wilson et al. (2017)	Subtropical N Pacific				33	
Wu et al. (2021)	Eastern Indian Ocean				48	7
Yogev et al. (2011)	E Mediterranean Sea				16	32[a]
Zhang et al. (2015)	South China Sea				82	11
Zhang et al. (2019)	Tropical NW Pacific				87	9[a]
Total		228	124	113	3735	1497

[a] Data are reported by data providers as depth-integrated N_2 fixation rates (unlabelled data computed from volumetric N_2 fixation rate data)

Table 3. Summary of data points of cell-count-based abundance in version 2 of the database, including volumetric
measurements for *Trichodesmium*, unicellular and heterocystous diazotrophs.

Reference	Region	Cell-count-based abundance				
		Tricho-desmium	Unicellular	*Richelia*	*Calothrix*	Depth-integrated
Bif and Yunes (2017)	S Atlantic	16				
Campbell et al. (2005)	SW Pacific					33[a]
Detoni et al. (2016)	S Atlantic	14				
Dupouy et al. (2011)	SW Pacific	18				
Fernández et al. (2010)	Global					40[a]
Hegde et al. (2008)	Bay of Bengal	135				
Holl et al. (2007)	N Atlantic					10[a]
Krupke et al. (2013)	N Atlantic		9			
Lory et al. (2022)	S Pacific	3				
Lu et al. (2018)	W Equatorial Pacific	2				
Martínez-Pérez et al. (2016)	Tropical N Atlantic		56			14
Masotti et al. (2007)	Southwestern Pacific	20				5
Mompeán et al. (2013)	N Atlantic					43[a]
Pierella Karlusich et al. (2021)	Global	46		46	35	
Riou et al. (2016)	N Atlantic		20			5
Sahu et al. (2017)	Bay of Bengal	14				
Shiozaki et al. (2013)	W Pacific	10		12		
Shiozaki et al. (2015a)	NW Pacific	60				10
Subramaniam et al. (2008)	N Atlantic					162[a]
White et al. (2018)	N Pacific	83		83		38
Wu et al. (2021)	Bay of Bengal	224		224		
Total		645	85	365	35	360

[a] Data are reported by data providers as depth-integrated cell-count-based abundance (unlabelled data computed from
volumetric cell-count-based abundance).

Table 4. Summary of data points of *nifH*-based abundance in version 2 of the database, including volumetric measurements
for *Trichodesmium*, unicellular and heterocystous diazotrophs.

References	Region	*nifH*-based abundances			
		Tricho-desmium	Unicellular	Heterocystous	Depth-integrated
Benavides et al. (2016a)	N Atlantic	13	30	15	
Bentzon-Tilia et al. (2015)	Baltic Sea		20		
Berthelot et al. (2017)	W Tropical Pacific	64	256	64	96
Bombar et al. (2011)	S China Sea	18	36	18	
Bombar et al. (2015)	N Pacific				32
Chen et al. (2019)	W Pacific	103	381	177	123
Cheung et al. (2020)	N Pacific	519	519		
Cheung et al. (2022)	Western Bering Sea		58	29	
Church and Zehr (2020)	N Pacific	968	1936	1936	605
Church et al. (2008)	N Pacific				60
Detoni et al. (2022)	WS Atlantic	70	140	70	72
Gradoville et al. (2020)	N Pacific	43	85	28	
Hallstrøm et al. (2022)	NE Atlantic				42[a]
Halm et al. (2012)	S Pacific Gyre	8	16		
Hamersley et al. (2011)	S California Bight	6	12	6	
Harding et al. (2018)	Arctic Ocean		39		
Hashimoto et al. (2016)	Seto Inland Sea		176		
Henke et al. (2018)	W Tropical S Pacific		142		
Krupke et al. (2013)	N Atlantic		24		3
Liu et al. (2020)	S China Sea	49	98		33
Lory et al. (2022)	W Tropical S Pacific	3	3		
Lu et al. (2018)	W Tropical Pacific	3	6	3	
Martínez-Pérez et al. (2016)	N Tropical Atlantic	84	252	84	70
Moisander et al. (2014)	S Pacific	174	348	174	92
Moore et al. (2018)	Tropical Atlantic	104	312	208	
Moreira-Coello et al. (2017)	N Pacific		20		20[a]

References	Region	nifH-based abundances			
		Tricho-desmium	Unicellular	Heterocystous	Depth-integrated
Ratten et al. (2015)	N Atlantic	9	27	9	10
Reeder et al. (2022)	Baltic Sea		15	15	
Sato et al. (2021)	Subarctic Sea		31		3
Shiozaki et al. (2014a)	Arabian Sea	26	52		18
Shiozaki et al. (2014b)	S China Sea	171	342		72[a]
Shiozaki et al. (2015a)	Temperate N Pacific	73	146		33
Shiozaki et al. (2017)	N Pacific	74	222	74	90
Shiozaki et al. (2018a)	Kuroshio	46	138	46	
Shiozaki et al. (2018b)	W Arctic		84		21
Shiozaki et al. (2018c)	S Pacific	94	285	95	95
Shiozaki et al. (2020)	Antarctic sea ice		53		
Sohm et al. (2011)	S Atlantic Gyre		58		
Stenegren et al. (2018)	W Tropical S Pacific	108	402	120	108
Tang et al. (2020)	N Atlantic	42	42		
Turk-Kubo et al. (2014)	E Tropical S Pacific	60	159	57	53
Wen et al. (2017)	W Pacific	22	44	22	
Wen et al. (2022)	W Pacific	130	390	130	110[a]
White et al. (2018)	N Pacific				34
Wu et al. (2019)	Bay of Bengal	68	63		19
Total		3082	7462	3281	1914

[a] Data are reported by data providers as depth-integrated *nifH*-based abundance (unlabelled data computed from volumetric *nifH*-based abundance).

2.2 Nitrogen fixation rates

Marine N_2 fixation rates are commonly measured using the acetylene reduction assay or $^{15}N_2$ assimilation method (Mohr et al., 2010; Montoya et al., 1996; Capone, 1993). The acetylene reduction assay estimates gross N_2 fixation rates indirectly by using a factor of 3:1 (Stewart et al., 1967; Bhavya et al., 2019) or 4:1 (Zehr and Capone, 2021) to convert acetylene reduction rates to N_2 fixation rates. When using the $^{15}N_2$ assimilation method, samples are incubated in seawater with $^{15}N_2$ labelled gas; the $^{15}N/^{14}N$ ratio of particulate nitrogen is measured at the beginning and at end of incubation to calculate the N_2 fixation rate

(Capone and Montoya, 2001). Compared to the $^{15}N_2$ assimilation method, the acetylene reduction assay is easier to conduct,

110   but acetylene used in the assay can potentially impact the metabolic activities of diazotrophs (Bhavya et al., 2019). The conversion factor between acetylene reduction and N_2 fixation is also controversial (Flett et al., 1976; Giller, 1987; Hardy et al., 1973). The $^{15}N_2$ assimilation method has higher sensitivity and requires a shorter incubation time than the acetylene reduction assay, particularly when N_2 fixation is low (Montoya et al., 1996). The $^{15}N_2$ assimilation method, however, needs to concentrate cells for signal detection, which can potentially damage cells and underestimate N_2 fixation rates (Bhavya et al.,

115   2019). Hence, 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).

     The conventional $^{15}N_2$ assimilation method was conducted by bubbling $^{15}N_2$-labelled gas. It was later found to be difficult to reach complete solubility equilibrium over a short incubation time, leading to serious underestimations of N_2 fixation rates

120   (Mohr et al., 2010; Großkopf et al., 2012; Wannicke et al., 2018). This "bubble" method was then modified by dissolving $^{15}N_2$-labelled gas in seawater for an adequate period to ensure that it reached solubility equilibrium (Mohr et al., 2010). Recently, this dissolution method was reported to have risks of introducing heavy metal pollution and affecting the growth of diazotrophs (White et al., 2020).

     We compared volumetric N_2 fixation rates in the upper 50 m and depth-integrated N_2 fixation rates in the database

125   measured using the above three methods, and found that they basically span a similar range of magnitude (**Fig. 1**). The results of a further analysis comparing the $^{15}N_2$ bubble and dissolution methods will be discussed later.

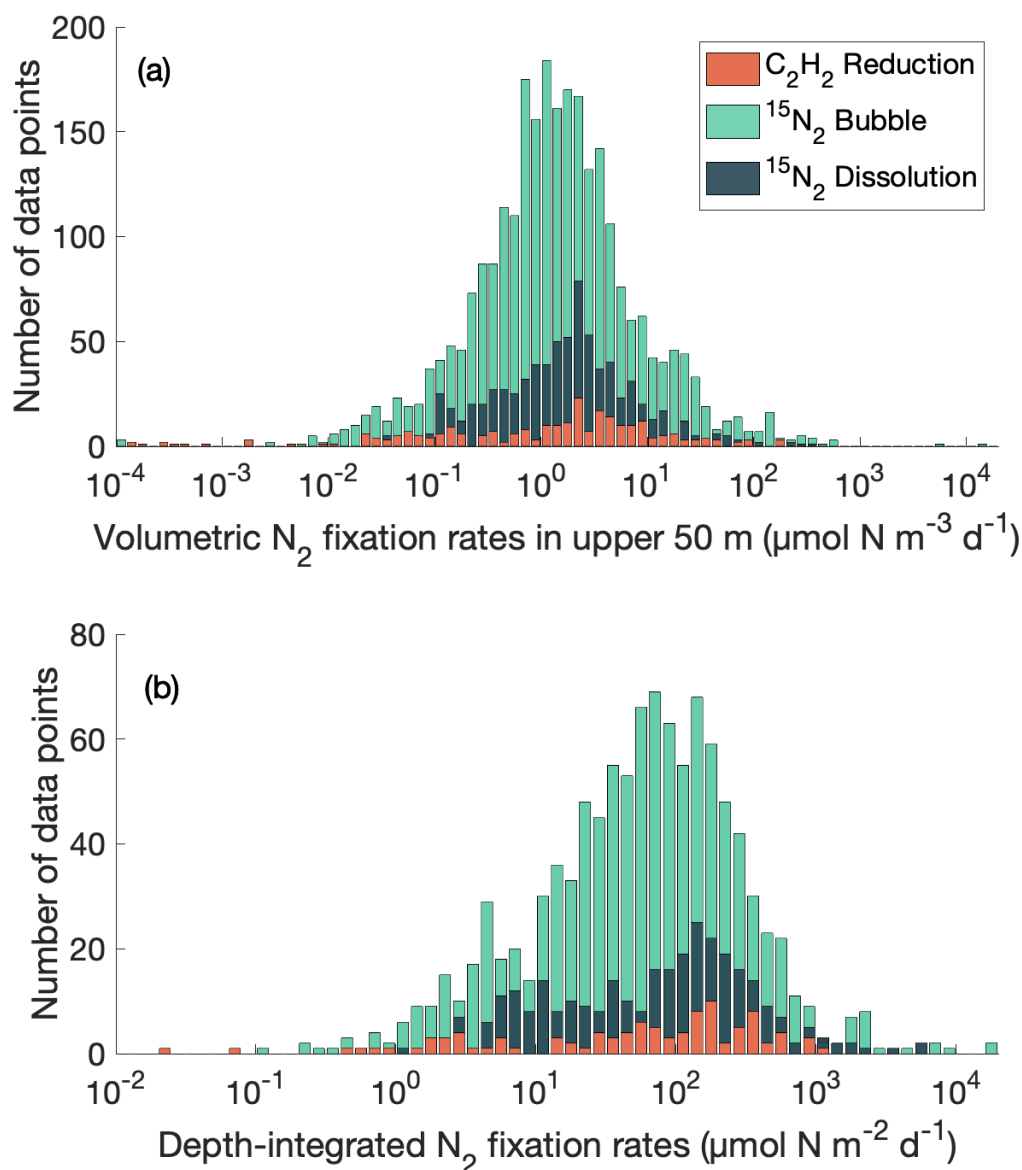
     Most N_2 fixation rates were originally reported as daily rates. For those reported hourly N_2 fixation rates in the daytime, we converted them to daily rates by multiplying by 12 hours. Some studies also reported hourly rates at night. We multiplied these night rates by 12 hours and added them to the daytime rates.

130

Figure 1. Distribution of N$_2$ fixation rates measured using different methods. (a) Volumetric data in upper 50 m; (b) depth-integrated data.

2.3 Diazotrophic abundance

Diazotroph cell abundance was counted mainly by using standard light microscopy, and in some cases by using

135   epifluorescence microscopy with the aid of color excitation. A recent study used machine learning techniques to detect and

enumerate diazotrophs in a large dataset of microscopic images (Pierella Karlusich et al., 2021). In the original database, only

the cell-counted abundance of *Trichodesmium* and heterocystous cyanobacteria was recorded. The updated database also

included a dataset of enumerated abundance of UCYN-A after staining them by CARD-FISH assays (**Table 3**) (Martínez-Pérez et al., 2016), although this unicellular diazotroph remained uncultivated.

140   *Trichodesmium* cell-count abundance in our database was recorded as the number of trichomes per volume of water, although it was also reported in the literature as the number of cells or colonies per volume of water. In the latter cases, the data were converted to trichomes per volume of water by using a commonly used factor of 200 trichomes colony^{-1} or 100 cells trichome^{-1} (Luo et al., 2012).

The abundance of heterocystous cyanobacterial cells was recorded in this database. When the number of symbioses was
145   reported in several studies, we assumed that 2 and 5 *Richelia* heterocystous cells were associated with each *Hemiaulus* and *Rhizosolenia* cell, respectively (Villareal et al., 2011), and 5 *Calothrix* heterocystous cells were associated with each *Chaetoceros* cell (Tuo et al., 2021).

In measurements of *nifH* copies, different qPCR assays were designed to target specific diazotrophic groups (Church et al., 2005; Foster et al., 2007), mainly including *Trichodesmium*, UCYN subgroups (A, B and C) and heterocystous groups
150   (**Table 4**). More recently, UCYN-A was found to have three sublineages, UCYN-A1, UCYN-A2, and UCYN-A3, with clade UCYN-A1 sharing the same genome as previously targeted UCYN-A (Thompson et al., 2014). UCYN-A1 and UCYN-A2 have significant distinctions in the sizes and species of their symbiotic hosts, with the former living in relatively smaller hosts (Thompson et al., 2014; Martínez-Pérez et al., 2016; Cornejo-Castillo et al., 2016). The *nifH* copies of these two sublineages were included in our database. The *nifH* copies of UCYN-A3 were not listed separately in our database because there were
155   only very few measurements reported. Three major associations of heterocystous groups were also marked for the *nifH* data, including *Richelia-Hemiaulus* (het-1), *Richelia-Rhizosolenia* (het-2) and *Calothrix-Chaetoceros* (het-3).

2.4 Quality control

The data of N$_2$ fixation rates and diazotrophic abundance in the database ranged by several orders of magnitude. Extremely high values usually occurred during algal blooms, and zero values indicated that diazotrophic activity was below detection or
160   true absence at the sampling time and stations. The data (nonzero value) were first logarithmically transformed and then analyzed for outliers, considering that they were likely log-normally distributed (Fig. S1-S6). For each parameter, we used Chauvenet's criterion to identify suspicious outliers whose probability of deviation from the means is lower than $1/2n$, where n is the number of data points (Glover et al., 2011). Because N$_2$ fixation rates and diazotroph abundances in the ocean can be extremely low, this filtering only applies to data on the high side. Although these outliers (labelled in database) may be true
165   values, we flagged them to remind users for caution. These outliers were also not used in our estimation of global ocean N$_2$ fixation rates.

3. Results

3.1 Data distribution

Version 2 of the database approximately doubled the number of N_2 fixation rate data in the original database, filling spatial
170 gaps particularly in the Indian and Southern Oceans (**Figs. 2a, 2b, 3a & 3b**). The number of depth-integrated N_2 fixation rate
data tripled, potentially providing more constraints on estimating global marine N_2 fixation (**Figs. 2b & 3b**). *NifH*-based
abundance consisted of the largest fraction of new data, mostly in the Pacific and Atlantic Oceans (**Figs. 2e, f & 3e, f**).
Compared to other parameters, a relatively moderate amount of new cell-count data was added and mainly distributed in the
Indian and Atlantic Oceans (**Figs. 2c, d & 3c, d**). Overall, there were limited data on N_2 fixation and diazotrophic abundance
175 in the Southern Ocean.

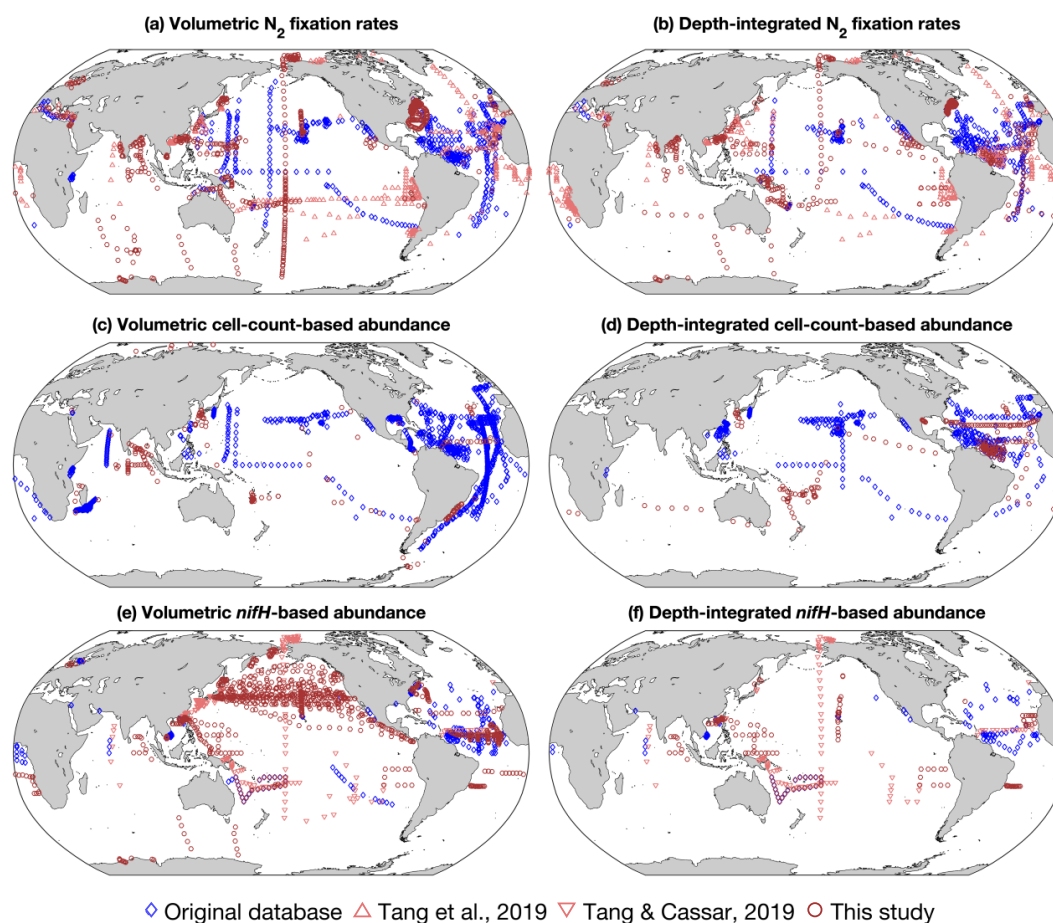



Figure 2. Spatial distribution of volumetric and depth-integrated data points in version 2 of the diazotrophic database binned
in 1°×1° grids. (**a-b**) N_2 fixation rates, (**c-d**) cell counts, and (**e-f**) *nifH* gene copies. The data sources include the original
version of this database (Luo et al., 2012) (blue diamonds), two compiled datasets (Tang et al., 2019; Tang and Cassar, 2019)
180 (red triangles) and this study (red circles).

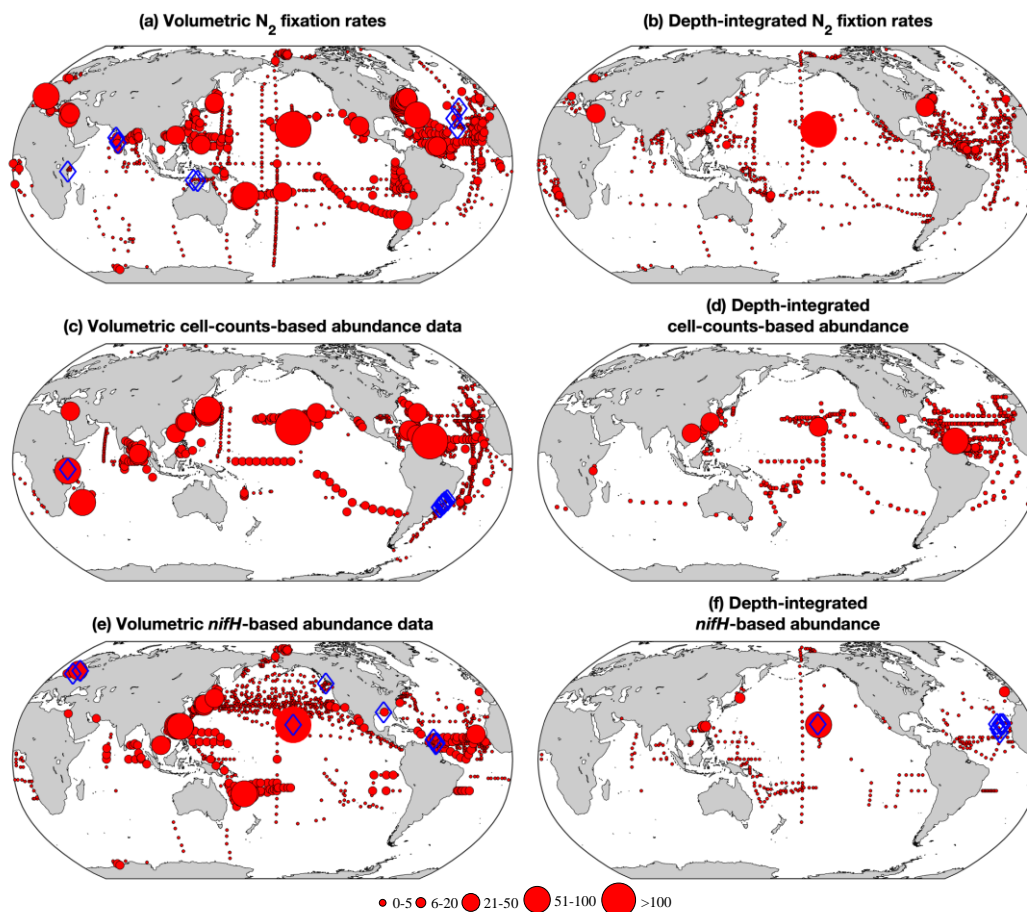



Figure 3. Spatial distribution of volumetric and depth-integrated data points binned in 1°×1° grids for (**a-b**) N$_2$ fixation rates, (**c-d**) cell counts, and (**e-f**) *nifH* gene copies. The size of the circles represents the number of data points in each bin. The blue diamonds mark the outliers identified using Chauvenet's criterion.

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Version 2 of the database added data at all latitudinal ranges (**Fig. 4**). In particular, version 2 extended the data from tropical and subtropical areas to polar regions in the Arctic Ocean (Harding et al., 2018) and Antarctic coast (Shiozaki et al., 2020).
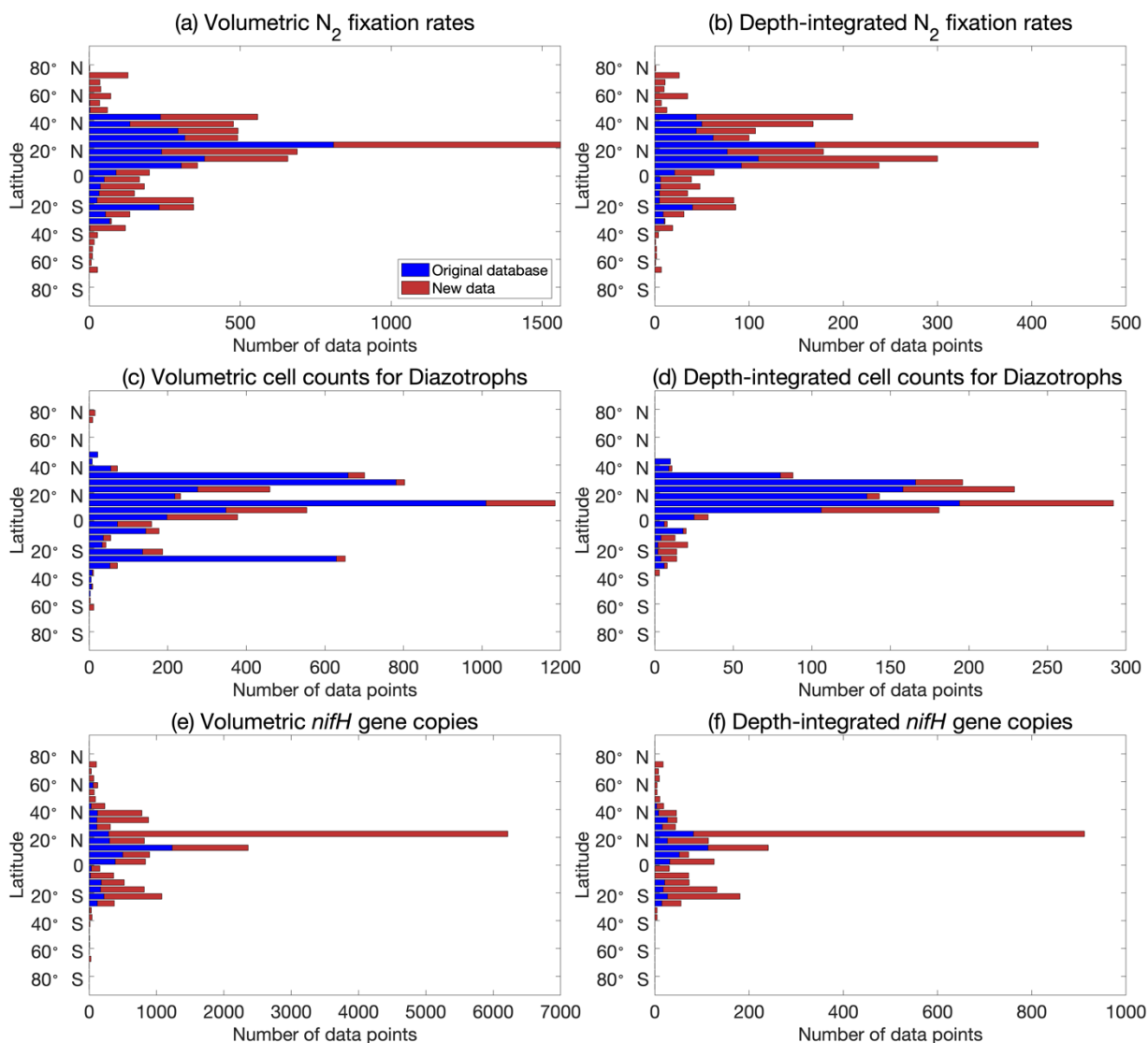
Figure 4. Latitudinal distribution of volumetric and depth-integrated data including (**a-b**) N$_2$ fixation rates, (**c-d**) cell counts, and (**e-f**) *nifH* gene copies, including the data in the original database (blue) and the new data added in version 2 of the database (red).

The data in version 2 of the database were distributed more evenly in months than in the original database, especially for the *nifH* copies, in which substantially more data were collected from January to February (**Fig. 5**).
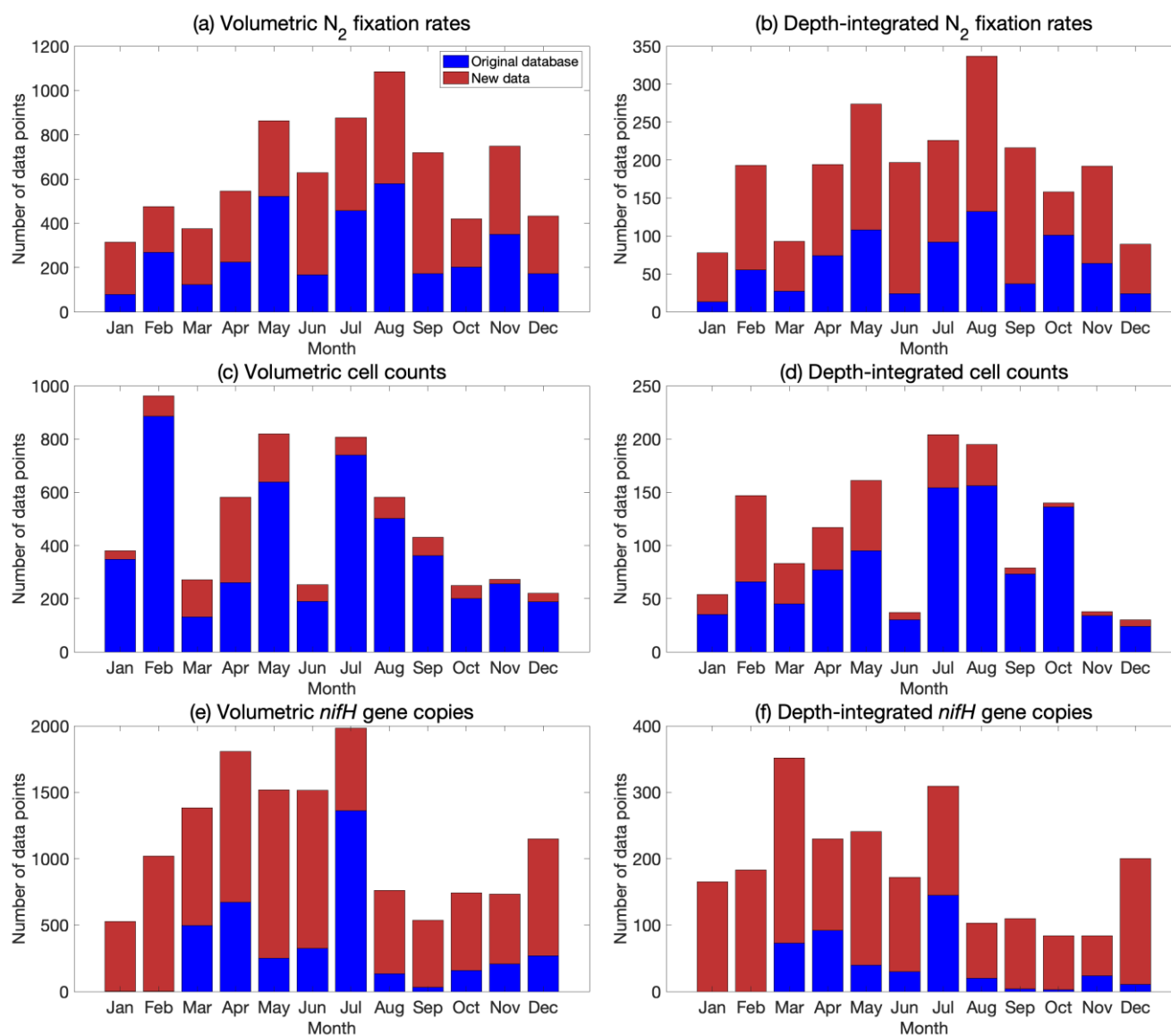
Figure 5. Temporal distribution of volumetric and depth-integrated data including (**a-b**) N$_2$ fixation rates, (**c-d**) cell counts, and (**e-f**) *nifH* gene copies, including the data in the original database (blue) and the new data added in version 2 of the database (red).

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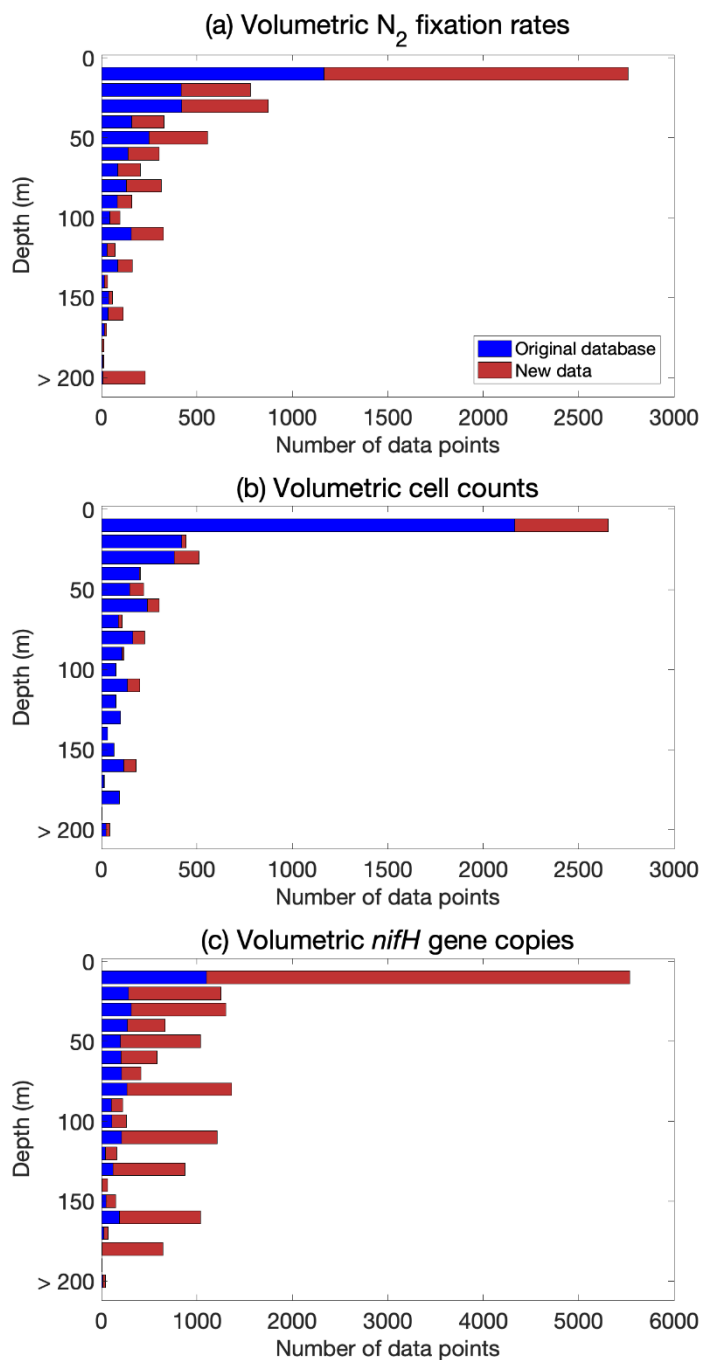
Although most of the new data were measured in surface waters, there were numerous *nifH* copy data sampled in other layers in the euphotic zone (**Fig. 6**). Additionally, active N$_2$ fixation and the existence of diazotrophs were found below the euphotic zone (e.g., depth > 200 m) (Benavides et al., 2016b; Benavides et al., 2018b; Selden et al., 2019; Hamersley et al., 2011; Bonnet et al., 2013; Loescher et al., 2014) (**Fig. 6**).

205

Figure 6. Vertical distribution of data including (**a**) N₂ fixation rates, (**b**) cell counts, and (**c**) *nifH* gene copies, including the data in the original database (blue) and the new data added in version 2 of the database (red).

3.2 N$_2$ fixation rates

The volumetric N$_2$ fixation rates in 5 vertical layers and the depth-integrated N$_2$ fixation rates were binned in 3°×3° grids, and the geometric means in each bin are displayed (**Fig. 7**). The depth-integrated N$_2$ fixation rates ranged in order of $10^{-4} - 10^3$ μmol N m^{-2} d^{-1} (mostly in order of $1 - 100$ μmol N m^{-2} d^{-1}) (**Fig. 7a**). Some high rates ($10^2 - 10^3$ μmol N m^{-2} d^{-1}) were found in the western Pacific Ocean, the regions near the Hawaii Islands, and the western tropical Atlantic Ocean. Approximately 25% of the depth-integrated N$_2$ fixation rates were lower than 1 μmol N m^{-2} d^{-1}, mainly in the North Atlantic Ocean and in the Indian Ocean. Vertically, the N$_2$ fixation rates were high in the upper 25 m (**Fig. 7b, c**), below which the rates rapidly decreased with depth (**Fig. 7d, e, f**). In the upper 25 m, the N$_2$ fixation rates in the southwestern Pacific were higher than those in other areas, mostly ranging from 1 to 100 μmol N m^{-3} d^{-1}. Note that some zero N$_2$ fixation rates were reported mostly in subpolar regions, as well as in certain tropical and subtropical regions (**Fig. 7**).

Depth-integrated N₂ fixation rates (µmol N m⁻² d⁻¹)

(a)

Volumetric N₂ fixation rates in 0 to 5 m (µmol N m⁻³ d⁻¹)

(b)

Volumetric N₂ fixation rates in 5 to 25 m (µmol N m⁻³ d⁻¹)

(c)

Volumetric N₂ fixation rates in 25 to 100 m (µmol N m⁻³ d⁻¹)

(d)

Volumetric N₂ fixation rates in 100 to 200 m (µmol N m⁻³ d⁻¹)

(e)

Volumetric N₂ fixation rates below 200 m (µmol N m⁻³ d⁻¹)

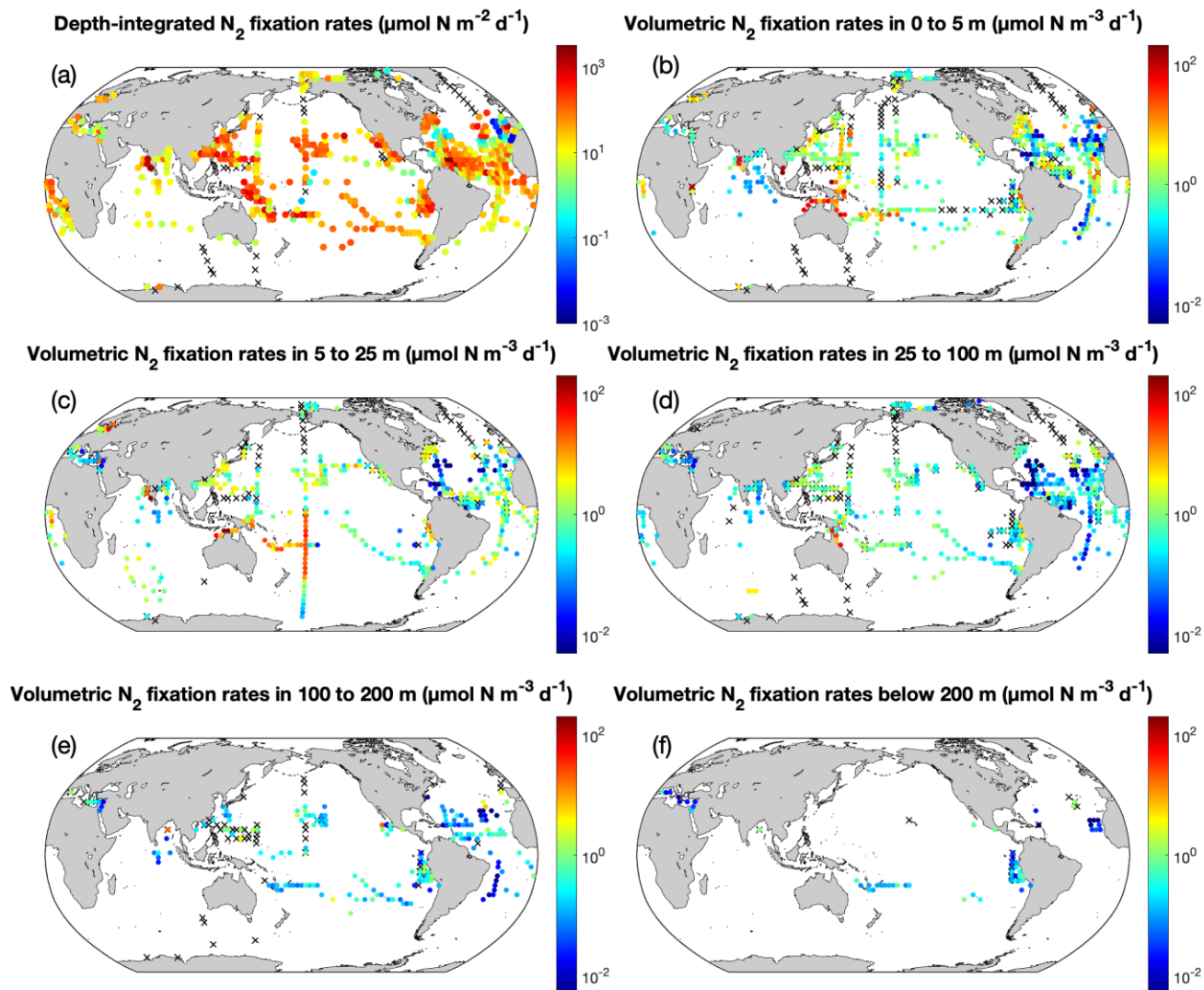
(f)

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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 geometric means in each bin are shown. Zero-value data are denoted as black crosses.

3.3 Diazotrophic abundance

225   The depth-integrated and upper 25 m volumetric cell-count-based abundance was also shown in geometric means of each 3° × 3° grid (**Fig. 8**). *Trichodesmium* abundance generally decreased from the west to the east in the Atlantic Ocean (**Fig. 8a–b**). In the Pacific Ocean, *Trichodesmium* appeared more abundant in the west. The abundance data of *Richelia* and *Calothrix* were still scarce (**Fig. 8c, e**).

The data of *nifH* copies had better spatial coverage than the cell-count data (**Fig. 9**). Depth-integrated *Trichodesmium nifH*
230   copies were also more abundant in the western Pacific and western Atlantic Oceans (**Fig. 9a**). Some high depth-integrated *nifH*
abundance of UCYN-A and UCYN-B was reported in the northwestern and southwestern Pacific Ocean (**Fig. 9c, e**). High
nifH abundance of *Richelia* was also found in the northwestern Pacific Ocean (**Fig. 9i**). The *nifH* abundance data for UCYN-
C and *Calothrix* were sparse. The volumetric *nifH* abundance data are also shown in four depth intervals (**Fig. 9 & Fig. S8**).
Almost all diazotrophs were more abundant in the upper 25 m than below.

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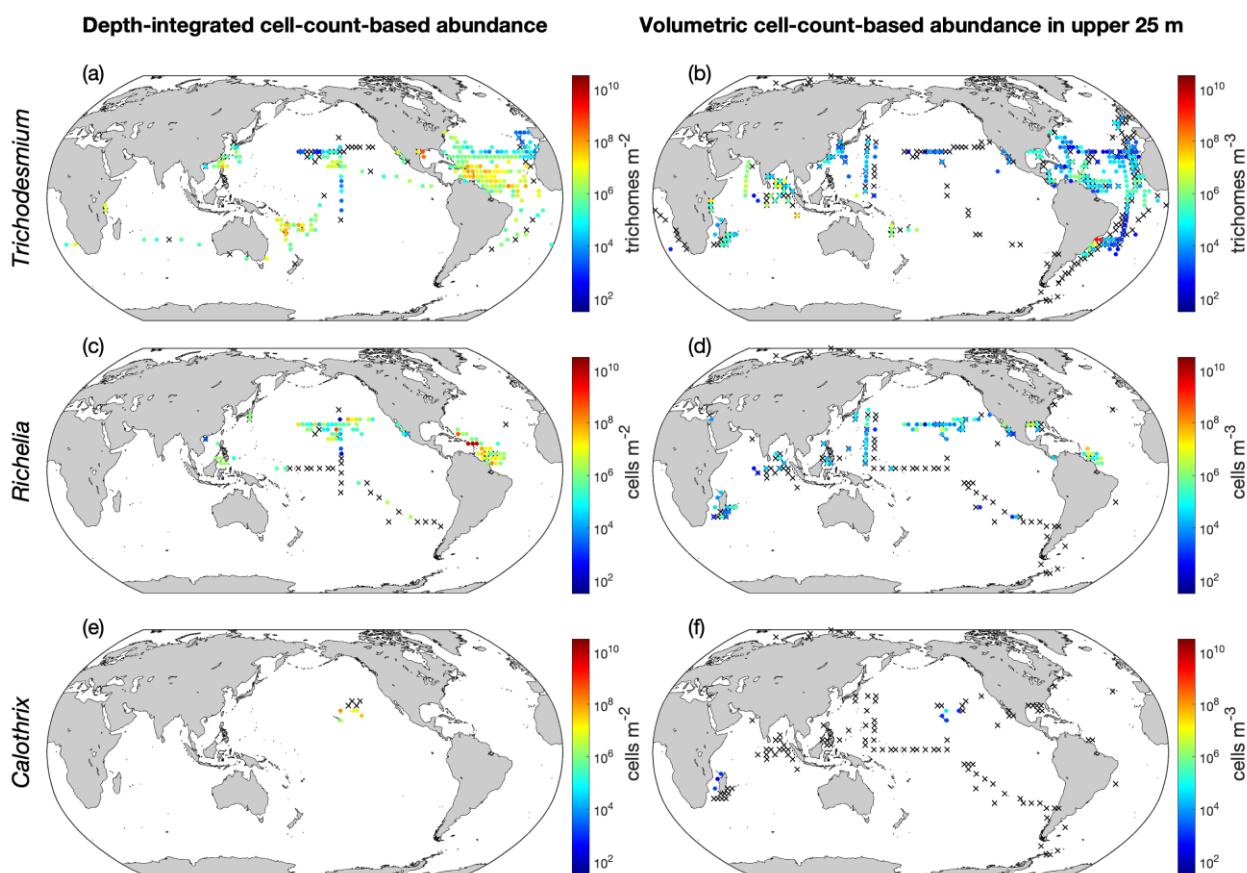
Figure 8. Depth-integrated cell-count-based abundance and upper 25 m volumetric cell-count-based abundance in version 2
of the database. The panels show (**a–b**) *Trichodesmium*, (**c–d**) *Richelia*, and (**e–f**) *Calothrix*. For a clear demonstration, data
are binned to 3° × 3°, and geometric means in each bin are shown. Zero-value data are denoted as black crosses. Note that the
240   abundance of *Trichodesmium* is reported as the number of trichomes per square or cubic meter.
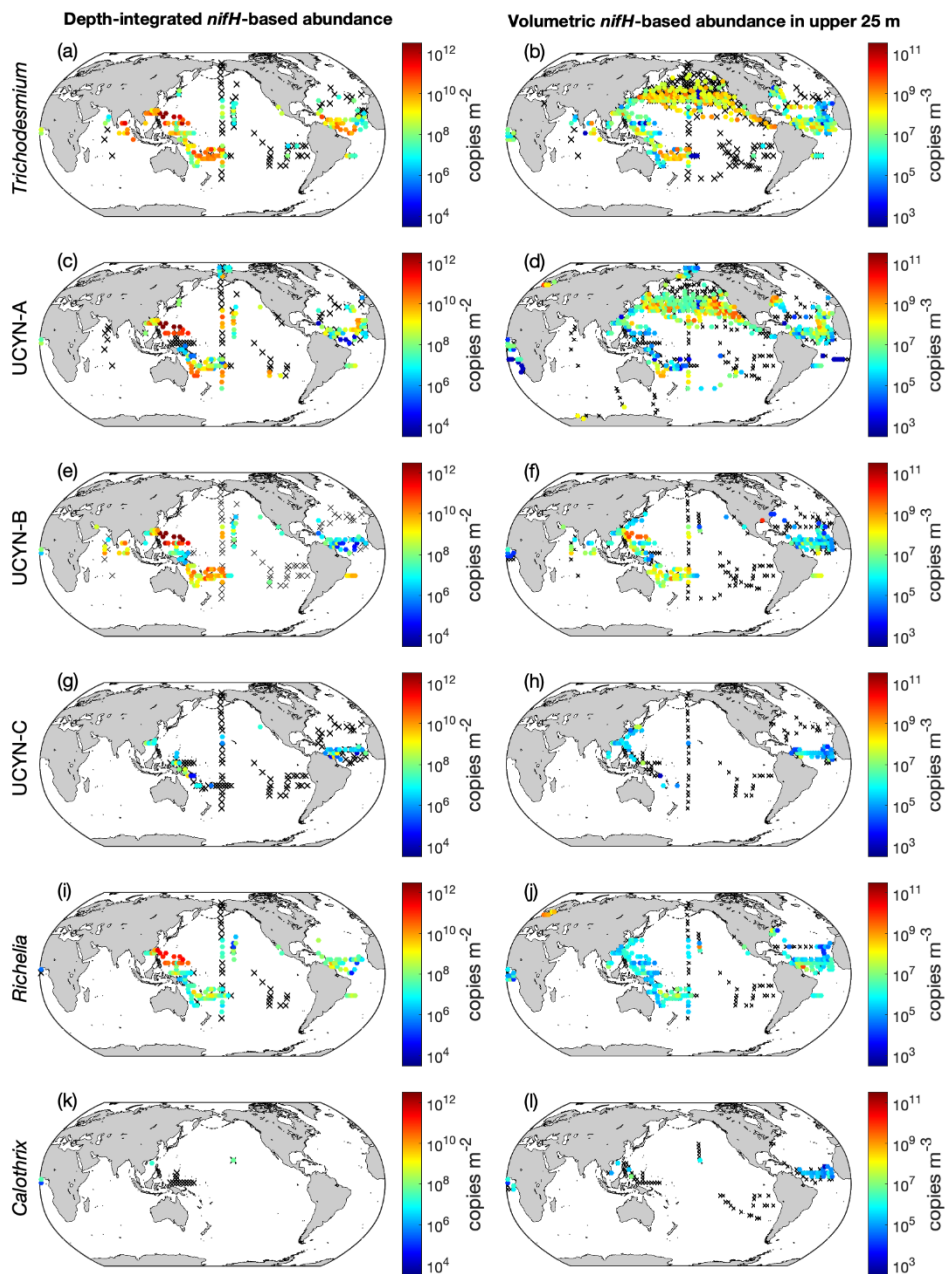
Figure 9. Depth-integrated *nifH* abundance and upper 25 m volumetric *nifH* abundance in version 2 of the database. The panels show (**a–b**) *Trichodesmium*, (**c–d**) UCYN-A, (**e–f**) UCYN-B, (**g–h**) UCYN-C, (**i–j**) *Richelia*, and (**k–l**) *Calothrix*. For a clear demonstration, data are binned to 3° × 3° and geometric means in each bin are shown. Zero-value data are denoted as black crosses.

3.4 First-order estimate of global ocean N$_2$ fixation rate

Similar to that applied to the original database, we used version 2 of the database to conduct a first-order estimate of global
250   ocean N$_2$ fixation rates (**Table 5**). The adequate data in version 2 supported the estimates of mean and total N$_2$ fixation rates
in the Indian and Arctic Oceans, while the estimates for these two ocean basins were not available when using the original
database (Luo et al., 2012). Version 2 estimated a much higher arithmetic mean of N$_2$ fixation rates in the South Pacific Ocean
than that using the original database, while the difference did not exist in the geometric means in the same basin. Another large
difference between the two versions of the database was the much lower geometric mean of N$_2$ fixation rates in the North
255   Pacific. The global oceanic N$_2$ fixation rate was calculated by summing its geometric or arithmetic means in every ocean basin,
with the associated errors being propagated (Glover et al., 2011; Luo et al., 2012). The estimates of global oceanic N$_2$ fixation
based on geometric means were close using the original and version 2 databases (62 and 60 Tg N yr^{-1}, respectively), while
those based on arithmetic means differed greatly (137 versus 260 Tg N yr^{-1}, respectively). This higher arithmetic mean-based
estimate of global oceanic N$_2$ fixation was mainly due to the supplementation of the Indian Ocean, for which the estimate was
260   unavailable when using the original database because of data limitations, and due to the higher rates in the South Pacific Ocean.
It must be noted that high uncertainties were associated with the arithmetic means of N$_2$ fixation in the South Pacific and Indian
Oceans when using version 2 (**Table 5**), indicating that more measurements were needed in these basins to better constrain the
elevated estimates of global oceanic N$_2$ fixation.

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Table 5. First-order estimates of N_2 fixation rates in different ocean basins. Data are first binned to $3°×3°$ grids before being used to calculate geometric or arithmetic means in each basin. The means are multiplied by the basin areas to calculate the N_2 fixation rates of each basin. NQ: not quantified due to limited data points. ND: no data. The numbers in parentheses after geometric means are confidence intervals estimated from one standard error of log-10 transformed data. Arithmetic means were reported with one standard error.

| Region | Number of binned data | | Mean N_2 fixation rate (μmol N m^{-2} d^{-1}) | | | | Areal sum (Tg N yr^{-1}) | | | |
| | | | Original database | | Version 2 | | Original database | | Version 2 | |
	Original database	Version 2	Geometric	Arithmetic	Geometric	Arithmetic	Geometric	Arithmetic	Geometric	Arithmetic
North Atlantic	125	180	9.2 (7.0−12)	170 ± 40	18.9 (18.6−19.1)	180 ± 33	1.7 (1.3−2.2)	32 ± 7.4	3.98 (3.93−4.03)	38 ± 7.0
South Atlantic	15	53	7.9 (6.3−9.8)	13 ± 4.4	17 (6.9−43)	30 ± 5	1.1 (0.9−1.4)	1.8 ± 0.6	2.7 (1.1−6.7)	4.6 ± 0.81
North Pacific	45	131	78 (67−90)	120 ± 22	50 (22−115)	130 ± 20	35 (30−41)	56 ± 9.8	20 (8.8−47)	52 ± 8.2
South Pacific	26	95	64 (54−76)	130 ± 46	58 (30−115)	240 ± 66	24 (20−28)	46 ± 17	20 (10−40)	83 ± 23
India Ocean	4	34	120 (29−490)	590 ± 320	17 (15−20)	270 ± 140	NQ	NQ	4.9 (4.3−5.6)	76 ± 33
Mediterranean Sea	10	12	18 (12−28)	45 ± 21	9.8 (7.4−13)	120 ± 94	0.2 (0.1−0.4)	0.6 ± 0.3	0.2 (0.15−0.26)	2.4 ± 1.9
Arctic Ocean	ND	17	ND	ND	14 (8.5−23)	19 ± 5	ND	ND	0.8 (0.48−1.3)	1.1 ± 0.28
Southern Ocean	ND	10	ND	ND	21 (7.7−58)	8.6 ± 8.1	ND	ND	6.5 (2.4−18)	2.7 ± 2.5
Global Ocean							62 (52−73)	137 ± 9.2	60 (47−107)	260 ± 20

4. Discussion

4.1 Comparison of N_2 fixation measured using $^{15}N_2$ bubbling and dissolution

Compared to the $^{15}N_2$ dissolution method, the magnitude of underestimation in N_2 fixation rates acquired by the $^{15}N_2$ bubble

275 method remains inconclusive. Here, we used data in our database to compare these two methods. First, as shown above, the volumetric N_2 fixation rates obtained by these two methods varied in a similar range of extent of magnitude (**Fig. 1**). The average N_2 fixation rate measured using the $^{15}N_2$ dissolution method was significantly higher (17%) than that measured using the $^{15}N_2$ bubble method (one-tailed Wilcoxon test, $p < 0.01$). We also compared N_2 fixation rates at the same location ($1° \times 1°$ grids) and months but measured by either the $^{15}N_2$ bubble or dissolution method, although the samples measured by the two

280 methods were not identical. The results showed that the $^{15}N_2$ dissolution method produced higher rates than the $^{15}N_2$ bubble method in 65% of cases (**Fig. 10**). The analysis using the generalized additive model (GAM) further demonstrated that the underestimation by the $^{15}N_2$ bubble method tended to be exaggerated under high N_2 fixation (> 3 µmol N m^{-3} d^{-1}) (**Fig. 10**), which can be explained by the gas equilibrium time (Mohr et al., 2010; Wannicke et al., 2018; Jayakumar et al., 2017): Under low N_2 fixation, the $^{15}N_2$ bubble method can provide sufficient dissolved $^{15}N_2$ regardless of whether the gas reaches equilibrium;

285 under high N_2 fixation, the $^{15}N_2$ bubble method cannot fulfil the requirement of dissolved $^{15}N_2$, resulting in relatively large underestimation.
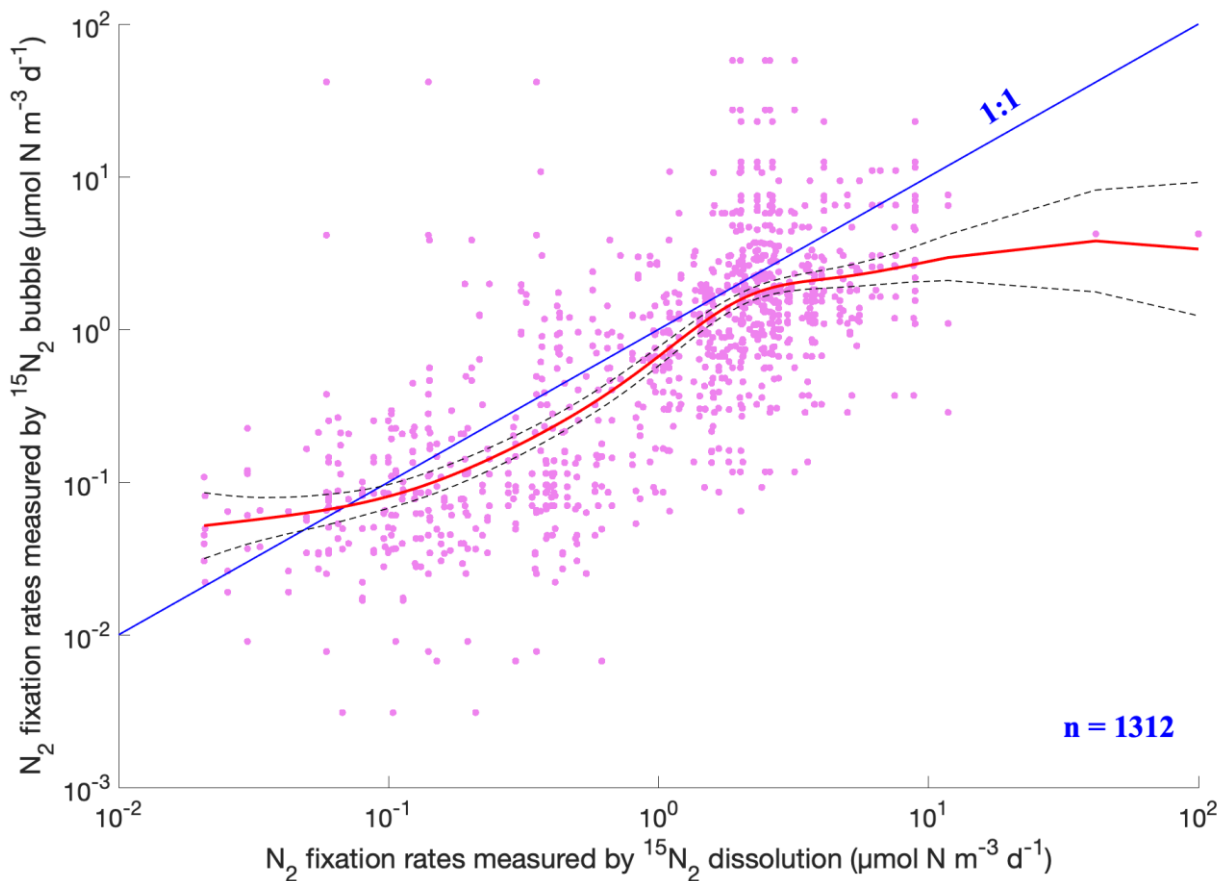
Figure 10. Comparison of measured N_2 fixation rates using the $^{15}N_2$ dissolution and the $^{15}N_2$ bubble assays. The blue line represents the 1:1 ratio of the two methods. The fitted results of the two methods by the generalized additive model (GAM) and confidence intervals are represented by the red solid line and the dashed black lines, respectively.

4.2 Comparison between diazotrophic cell counts and *nifH* copies

Whether *nifH* copies can be used to infer diazotrophic abundance remains debated, as a large range in the number of *nifH* copies per diazotrophic cell has been reported (**Table S1**). The reported ratios of *nifH* copies to cell numbers in *Trichodesmium* and heterocystous cyanobacteria appeared larger than those in UCYNs (**Table S1**), possibly caused by large genome size in *Trichodesmium* (Sargent et al., 2016) and inclusion of the *nifH* gene in vegetative cells of heterocystous filaments (White et al., 2018). In our database, the log-10 transformed abundance of *Trichodesmium* cell counts ($10^{6.5\pm1.2}$ cells L^{-1}) was only slightly lower than the log-10 transformed abundance of *Trichodesmium nifH* copies ($10^{6.6\pm1.6}$ copies L^{-1}) (**Fig. 11a**), while the difference was approximately two orders of magnitude in heterocystous cyanobacteria *Richelia* and *Calothrix* (**Fig. 11b, c**). It must be noted that this simple analysis used all the data in our database, and the cell counts and *nifH* copies were measured for samples that were not in the same season and location. Much lower ratios of cell counts to *nifH* copies ($1.51 - 2.58$) were

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recently reported in heterocystous cyanobacteria and UCYN-B collected near the Hawaii Islands (Gradoville et al., 2022), demonstrating potentially large variance in these ratios.
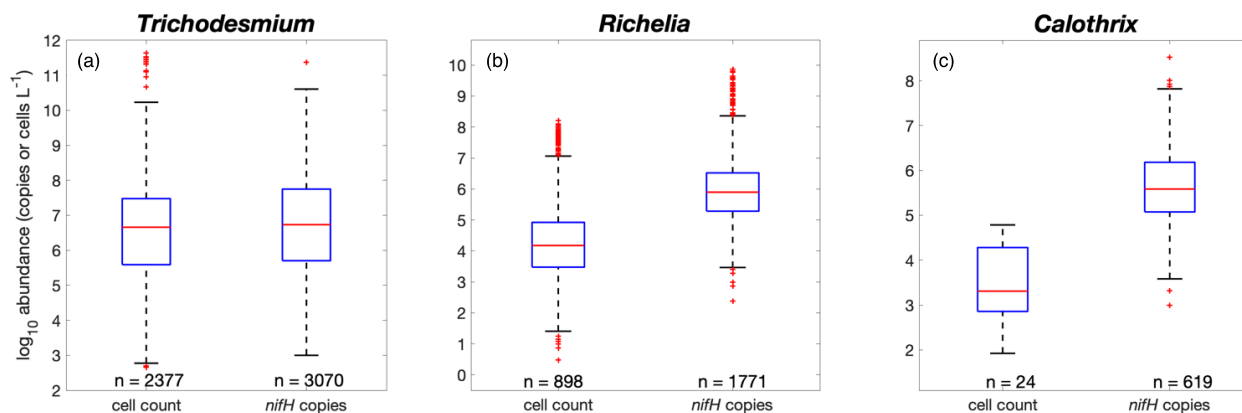
Figure 11. Comparison of all cell-count and *nifH* copy abundance data in the database. The box plots show the median (central line), 25th and 75th percentiles of log-10 transformed data (upper and lower edges of the boxes), 5th and 95th percentiles (error lines) and outliers (red crosses).

4.3 Biomass conversion factor

For the possible further use of cell-count or *nifH*-based abundance, here we suggest factors to convert the abundance to carbon biomass (**Tables 6 and S2**). Most biomass conversion factors suggested here are the same as those used in Luo et al. (2012), except for heterocystous cyanobacteria and UCYN-A because new information has become available or additional consideration is necessary.

Because heterocystous cyanobacteria and their host diatoms form DDAs and need to function together, we suggest allocating the biomass of host diatoms to each associated diazotrophic cell (**Table S2**). The carbon biomass of host diatom cells was calculated using an empirical equation (Menden-Deuer and Lessard, 2000):

$$C = 0.117 \times V^{0.881}, \tag{1}$$

where C is the diatom cell carbon biomass (pg C cell[-1]), and V is the average cell biovolume (μm^3) of each diatom genus, for which values from a database (Harrison et al., 2015) were used in this study (**Table S2**). Each host diatom associates with multiple heterocysts. The numbers of *Richelia* heterocysts in *Hemiaulus* and *Rhizosoleni* were observed to be 2 and 5 (Villareal et al., 2011; Yeung et al., 2012), and the number of vegetative cells in each heterocyst can range from 3 to 10 (Foster et al., 2011). We tried 2 and 5 *Calothrix* heterocysts in *Chaetocer*os when estimating the biomass conversion factor, although the values were unknown. The biomasses of heterocystous cells and vegetative cells (2–80 pg C cell[-1] for *Richelia* and 5–20 pg C cell[-1] for *Calothrix*) were adopted from Luo et al. (2012). Hence, the conversion factors for DDAs are estimated by dividing the total biomass of each DDA by the number of associated heterocysts. As a result, the biomass conversion factors of *Richelia-Hemiaulus* and *Richelia-Rhizosolenia* were estimated to be 280 (range: 150–1250) and 430 (range: 10–1900) pg C heterocyst[-]

325   [1], respectively (**Table S2**). As the number of filaments (2 or 5) did not have a large impact on the factors of *Calothrix-Chaetoceros* associations, we recommend using 100 (range: 20–360) pg C heterocyst[-1].

The conversion factor for UCYN-A is also updated because it has been found to live symbiotically with prymnesiophyte or coccolitophore species (Thompson et al., 2012). Similar to DDAs, the host biomass is allocated to UCYN-A. It has been reported that each prymnesiophyte cell hosts one UCYN-A1 cell, and each coccolitophore hosts 5–10 UCYN-A2 cells

330   (Cornejo-Castillo et al., 2019). The biomass of a UCYN-A1 cell with a diameter of 1 μm and a UCYN-A2 cell with a diameter of 1.6–3.3 μm (Cornejo-Castillo et al., 2019; Martínez-Pérez et al., 2016) is 0.2 and 0.8–5.5 pg C, respectively, by using an empirical equation (Verity et al., 1992):

$$C = 0.433 \times V^{0.863}, \tag{2}$$

Using Equation (2), the biomass of a host prymnesiophyte or coccolitophore cell is 1.5–2.2 pg C or 6.8–43 pg C according

335   to their reported cell diameters (2–2.3 μm and 3.6–7.3 μm, respectively) (Martínez-Pérez et al., 2016; Cornejo-Castillo et al., 2019). Hence, the biomass of the UCYN-A1 symbiosis and the UCYN-A2 symbiosis is 1.7–2.4 pg C and 7.6–48 pg C, respectively. After normalizing the symbiotic biomass to the number of UCYN cells in each symbiosis (1 for UCYN-A1 and 5–10 for UCYN-A2), the biomass conversion factors are 1.7–2.4 pg C (UCYN-A1 cell)[-1] and 0.8–9.6 pg C (UCYN-A2 cell)[-1]. Thus, we recommend using a uniform conversion factor of 2 pg C cell[-1] for these two clades of UCYN-A (**Table 7**)

340   considering that UCYN-A1 is more frequently found and often in higher abundance than UCYN-A2 (Thompson et al., 2014).

Table 6. Biomass conversion factors for diazotrophs.

	Trichodesmium (pg C cell⁻¹)	UCYN-A (pg C cell⁻¹)	UCYN-B (pg C cell⁻¹)	UCYN-C (pg C cell⁻¹)	*Richelia-Hemiaulus* (pg C heterocyst⁻¹)	*Richelia-Rhizosolenia* (pg C heterocyst⁻¹)	*Calothrix-Chaetoceros* (pg C heterocyst⁻¹)
Recommended	300	2	20	10	280	430	100
Range	100−500	1−10	4−50	5−24	150−1250	10−1900	20−360

5. Conclusions

This study updated the global oceanic diazotrophic database by Luo et al. (2012) using new measurements reported in the past decade. Although the spatial coverage of the data was greatly expanded by this effort, the data distribution is still uneven, with most measurements in the Pacific and Atlantic Oceans. The estimation of global oceanic N_2 fixation based on its arithmetic means in ocean basins was greatly increased by using the updated database, particularly in the Indian and South Pacific Oceans. Although this result may suggest the potential to reduce the imbalance existing in estimated N_2 fixation and N removal rates in the global ocean, large uncertainties still exist, and better constraints with more measurements are needed in the future. For instance, if geometric means of N_2 fixation in ocean basins were instead used, the updated database did not increase the estimation of global oceanic N_2 fixation. Furthermore, data were more concentrated in surface seawater, especially in the Southern Ocean, limiting reliable evaluations of depth-integrated N_2 fixation rates. We also compared the N_2 fixation rates measured using bubbling or dissolving $^{15}N_2$ gases at the same location and month (not necessarily in identical samples). The results showed that the bubbling method produced lower rates, on average, than the dissolution method, although the former method even generated higher rates in approximately one-third of cases. All these results suggest that, despite the efforts in the past several decades, the ocean is still under sampled in terms of the distribution of diazotrophs and level of N_2 fixation. Nevertheless, we believe this diazotrophic database, updated with a large amount of data accumulated in the past decade, is timely and can help scientists study marine ecology and biogeochemistry.

Data availability.

The database is available in a data repository (https://doi.org/10.6084/m9.figshare.21677687) (Shao et al., 2022)

Author contributions.

YWL conceived and designed the structure of the database and supervised the study. All the authors collected the data and updated the database. ZS, YX, HW and YWL analyzed the data. ZS, YX and YWL wrote the manuscript.

Competing interests.

The authors declare that they have no conflicts of interest.

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