Evidence of high N₂ fixation rates in productive waters of the temperate Northeast Atlantic

Debany Fonseca-Batista*, Xuefeng Li, Virginie Riou, Valérie Michotey, Florian Deman, François Fripiat, Sophie Guasco, Natacha Brion, Nolwenn Lemaitre, Manon Tonnard, Morgane Gallinari, Hélène Planquette, Frédéric Planchon, Géraldine Sarthou, Marc Elskens, Lei Chou, Frank Dehairs

* Correspondence:

Debany Fonseca-Batista:

dbatista8@hotmail.com

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Supplementary Figure S1. Neighbour-joining phylogenetic tree of *nifH* predicted amino acid sequences generated using the Molecular Evolutionary Genetics Analysis software (MEGA 7.0) [Kumar et al., 2016]. Sequences for representative clones ($\geq 95\%$ identity at the nucleotide level) are given in bold (proportion of sequences at stations Bel-11 and Bel-13 are indicated in parenthesis). Bootstrap support values ($\geq 70\%$) for 100 replications are shown at nodes. The scale bar indicates the number of sequence substitutions per site. The archaean *Methanobrevibacter smithii* was used as an outgroup. Accession numbers for published sequences used to construct the phylogenetic tree are given.

Supplementary Table S1. Summary of the dataset used to run principal component analyses relating volumetric rates of N_2 fixation and primary production to environmental variables

Station	Date	Depth [m]	Lat. [°N]	Long. [°E]	N ₂ fixation [nmol N L ⁻¹ d ⁻¹]	Primary production [µmol C m ⁻³ d ⁻¹]	Temperature [°C]	Salinity [psu]	NH4 ⁺ [μM]	NO ₃ ⁻ +NO ₂ ⁻ [μM]	Ρ* [μM]	Chl <i>a</i> [µg L ⁻¹]
Bel-3	24-May-2014	5	46.5	-8.0	< DL	1180.8	13.86	35.58	< DL	0.27	0.04	1.42
Bel-3	24-May-2014	10	46.5	-8.0	< DL	776.1	13.87	35.58	< DL	0.36	0.04	1.25
Bel-3	24-May-2014	25	46.5	-8.0	< DL	995.9	13.78	35.58	< DL	1.14	0.06	1.10
Bel-3	24-May-2014	50	46.5	-8.0	< DL	109.7	12.44	35.62	0.35	4.62	-0.03	0.16
Bel-5	25-May-2014	5	45.3	-8.8	< DL	429.1	13.91	35.59	0.19	0.66	0.02	0.16
Bel-5	25-May-2014	30	45.3	-8.8	< DL	360.6	13.91	35.59	0.59	0.91	0.00	0.12
Bel-5	25-May-2014	70	45.3	-8.8	< DL	433.9	13.24	35.61	0.59	4.71	-0.04	0.21
Bel-5	25-May-2014	130	45.3	-8.8	< DL	9.7	11.92	35.62	< DL	7.74	-0.14	0.00
Bel-7	26-May-2014	5	44.6	-9.3	1.1	849.1	13.94	35.51	< DL	< DL	0.05	1.19
Bel-7	26-May-2014	16	44.6	-9.3	1.0	707.3	13.94	35.52	< DL	0.24	0.05	1.14
Bel-7	26-May-2014	30	44.6	-9.3	2.0	1018.3	13.86	35.52	0.09	1.01	0.00	0.98
Bel-7	26-May-2014	80	44.6	-9.3	1.6	70.2	13.32	35.55	0.34	2.84	0.02	0.16
Geo-21	31-May-2014	10	46.5	-19.7	8.2	2824.8	14.57	35.68	0.18	1.52		0.88
Geo-21	31-May-2014	18	46.5	-19.7	< D L	3443.7	13.70	35.69	0.39	2.21		1.21
Geo-21	31-May-2014	25	46.5	-19.7	4.9	3500.1	13.47	35.68	0.50	2.82		0.73
Geo-21	31-May-2014	40	46.5	-19.7	1.4	1155.1	12.84	35.65	0.68	4.13		0.39
Geo-21	31-May-2014	60	46.5	-19.7	2.0	393.8	12.84	35.69	0.41	5.32		0.19
Geo-21	31-May-2014	91	46.5	-19.7	2.3	73.8	12.51	35.70	< DL	7.19		

Supplementary Table S1 continued.

Station	Date	Depth [m]	Lat. [°N]	Long. [°E]	N ₂ fixation [nmol N L ⁻¹ d ⁻¹]	Primary production [µmol C m ⁻³ d ⁻¹]	Temperature [°C]	Salinity [psu]	$\mathrm{NH_4^+}$ [$\mu \mathrm{M}$]	NO ₃ ⁻ +NO ₂ ⁻ [μM]	Ρ* [μM]	Chl a [µg L ⁻¹]
Bel-9	27-May-2014	5	42.4	-9.7	3.9	335.9	16.04	35.55	< DL	< DL	0.05	0.16
Bel-9	27-May-2014	25	42.4	-9.7	0.7	207.3	15.96	35.56	< DL	0.38	0.04	0.18
Bel-9	27-May-2014	45	42.4	-9.7	0.9	571.2	14.33	35.83	< DL	2.07	-0.01	0.55
Bel-9	27-May-2014	120	42.4	-9.7	< DL	6.9	13.24	35.78	< DL	5.99	-0.08	0.01
Bel-11	28-May-2014	5	40.7	-11.1	65.4	565.3	16.95	35.56	< DL	< DL	0.05	0.15
Bel-11	28-May-2014	35	40.7	-11.1	7.0	630.7	15.18	35.84	< DL	< DL	0.05	0.28
Bel-11	28-May-2014	45	40.7	-11.1	< DL	292.3	15.26	35.83	< DL	0.61	0.02	0.12
Bel-11	28-May-2014	80	40.7	-11.1	4.9	334.6	14.01	35.90	< DL	4.35	-0.08	0.23
Bel-13	29-May-2014	5	38.8	-11.4	45.0	599.4	17.23	35.68	< DL	< DL	0.05	0.22
Bel-13	29-May-2014	30	38.8	-11.4	10.5	323.2	16.46	35.89	< DL	< DL	0.09	0.09
Bel-13	29-May-2014	45	38.8	-11.4	12.6	692.0	15.38	35.97	< DL	0.52	0.12	0.20
Bel-13	29-May-2014	80	38.8	-11.4	2.4	92.6	14.84	36.10	< DL	2.39	0.07	0.08
Geo-1	19-May-2014	6	40.3	-10.0	4.8	621.8	16.70	35.11	0.33	< DL		0.16
Geo-1	19-May-2014	11	40.3	-10.0	7.1	696.9	16.53	35.18	< DL	< DL		0.19
Geo-1	19-May-2014	16	40.3	-10.0	4.8	667.8	16.09	35.26	< DL	< DL		
Geo-1	19-May-2014	25	40.3	-10.0	2.5	579.1	15.33	35.36	< DL	0.42		0.33
Geo-1	19-May-2014	34	40.3	-10.0	1.2	842.3	15.14	35.46	< DL	0.76		0.35
Geo-1	19-May-2014	48	40.3	-10.0	1.1	676.9	14.35	35.62	< DL	2.44		0.46
Geo-2	20-May-2014	11	40.3	-9.5	4.7	474.3	16.82	34.99	< DL	< DL		0.21
Geo-2	20-May-2014	31	40.3	-9.5	2.8	1170.5	14.67	35.54	< DL	0.93		0.47
Geo-2	20-May-2014	39	40.3	-9.5	2.7	1149.5	13.97	35.70	0.09	1.38		0.98
Geo-2	20-May-2014	85	40.3	-9.5	2.3	72.5	13.32	35.77	< DL	4.39		0.02

Supplementary Table S1 final.

Station	Date	Depth [m]	Lat. [°N]	Long. [°E]	N ₂ fixation [nmol N L ⁻¹ d ⁻¹]	Primary production [µmol C m ⁻³ d ⁻¹]	Temperature [°C]	Salinity [psu]	NH4 ⁺ [μM]	NO ₃ ⁺ HO ₂ ⁻ [µM]	Ρ* [μM]	Chl a [µg L ⁻¹]
Geo-13	24-May-2014	15	41.4	-13.9	5.5	1670.7	15.47	35.84	0.21	0.34		0.56
Geo-13	24-May-2014	30	41.4	-13.9	1.0	403.0	14.73	35.81	0.34	0.68		0.68
Geo-13	24-May-2014	43	41.4	-13.9	2.4	910.9	13.66	35.77	0.73	2.21		
Geo-13	24-May-2014	58	41.4	-13.9	2.2	790.5	13.36	35.76	0.68	3.47		0.45
Geo-13	24-May-2014	75	41.4	-13.9	3.9	338.1	13.14	35.76	0.07	4.54		0.17
Geo-13	24-May-2014	116	41.4	-13.9	3.1	22.8	12.91	35.75	< DL	6.27		0.01

Supplementary Table S2. Summary of the dataset used to run principal component analyses relating depth-integrated rates of N_2 fixation and primary production to environmental variables.

Station	MLD	Lat. [°N]	Long. [°E]	N ₂ fixation [µmol N m ⁻² d ⁻¹]	Primary Production [mmol C m ⁻² d ⁻¹]	Euphotic layer averaged Temperature [°C]	Euphotic layer averaged Salinity [psu]	Euphotic layer integrated Chl <i>a</i> [mg m ⁻²]	Euphotic layer integrated NH4 ⁺ [µmol m ⁻²]	Euphotic layer integrated NO ₃ ⁻ +NO ₂ ⁻ [µmol m ⁻²]	climatology P* at 20 m depth [µmol m ⁻²]	Dust deposition [Apr. 2014] [µg m ⁻² d ⁻¹]	Dust deposition [May 2014] [µg m ⁻² d ⁻¹]
Bel-3	27	46.5	-8.0	0	37.9	13.5	35.6	47.1	6.7	86.2	0.06	1263	2539
Bel-5	41	45.3	-8.8	0	42.6	13.2	35.6	17.1	54.0	509.0	0.06	1647	1914
Bel-7	33	44.6	-9.3	128	52.1	13.8	35.5	62.3	12.9	107.1	0.07	2147	1443
Geo-21	17	46.5	-19.7	279	135.3	13.3	35.7	38.1	34.2	452.5	0.04	2147	1443
Bel-9	26	42.4	-9.7	81	36.6	14.9	35.7	32.6	7.7	332.2	0.06	3650	1088
Bel-11	33	40.7	-11.1	1533	36.4	15.3	35.8	15.2	5.1	93.5	0.05	2799	618
Bel-13	25	38.8	-11.4	1355	35.9	16.0	35.9	12.1	5.1	58.3	0.06	2147	618
Geo-1	12	40.3	-10.0	141	33.1	15.7	35.3	14.2	5.3	68.4	0.03	3650	618
Geo-2	14	40.3	-9.5	262	59.1	14.7	35.5	37.8	6.0	219.4	0.01	4758	820
Geo-13	29	41.4	-13.9	384	78.9	13.9	35.8	42.4	34.0	274.2	0.07	1647	820

Supplementary Table S3. Spearman correlation matrix opposing depth-integrated rates of N₂ fixation and primary production (PP), from BG2014/14 and GEOVIDE cruises together, to euphotic layer averaged or integrated environmental variables. The correlation factor (r) and its significance given by the p-value (p) at p < 0.001, p < 0.01 and p < 0.05 presented with ***, ** and *, respectively, and the number of observations (n) are shown for each combination tested. Also, dFe correlations were made only for GEOVIDE sampling stations.

		Average salinity	Integrated $[NH_4^+]$ [µmol m ⁻²]	Integrated $[NO_3 + NO_2]$ $[\mu mol m^2]$	Average in situ P* [µM]	Surface climatology P* [µM]	Integrated DFe [nmol m ⁻²]	Dust deposition April 2014 (kg m ⁻² s ⁻¹)	Dust deposition May 2014 (kg m ⁻² s ⁻¹)	$\frac{PP}{[mmol m^2 d^{-1}]}$	N_2 fixation [µmol m ⁻² d ⁻¹]
Average	r	0.188	-0.869***	-0.721*	0.0788	-0.0732	0.8	0.587	-0.901***	-0.721*	0.553
temperature	р	0.583	0.0000002	0.0157	0.811	0.811	0.333	0.0665	0.0000002	0.0157	0.0892
[°C]	n	10	10	10	10	10	4	10	10	10	10
Average			-0.109	0.152	0.00606	0.366	-0.6	-0.29	-0.265	-0.0182	0.62*
salinity			0.733	0.656	0.973	0.275	0.417	0.404	0.446	0.946	0.048
			10	10	10	10	4	10	10	10	10
Integrated				0.857***	-0.0304	0.26	-0.8	-0.471	0.737*	0.729*	-0.482
$[\mathrm{NH}_4^+]$				0.0000002	0.919	0.446	0.333	0.16	0.0131	0.0131	0.148
[µmol m ⁻²]				10	10	10	4	10	10	10	10
Integrated					-0.0182	0.116	-0.8	-0.228	0.512	0.758**	-0.195
$[NO_3 + NO_2]$					0.946	0.733	0.333	0.512	0.116	0.0087	0.559
[µmol m ⁻²]					10	10	4	10	10	10	10
Average						-0.274	-0.4	0.161	-0.253	0.479	0.511
in situ P*						0.425	0.75	0.631	0.468	0.148	0.116
[µM]						10	4	10	10	10	10
Surface							-0.8	-0.491	0.205	0.0732	-0.0856
climatology P*							0.333	0.137	0.559	0.811	0.785
[µM]							4	10	10	10	10
Integrated								0.8	-0.632	-0.8	-0.6
DFe								0.333	0.333	0.333	0.417
[nmol m ⁻²]								4	4	4	4
Dust deposition									-0.588	-0.265	0.257
April 2014									0.0665	0.446	0.446
$(\text{kg m}^{-2} \text{ s}^{-1})$									10	10	10
Dust deposition										0.512	-0.774**
May 2014										0.116	0.00686
$(\text{kg m}^{-2} \text{ s}^{-1})$										10	10
PP											-0.0365
$[\text{mmol C m}^2 \text{ d}^1]$											0.892
											10

Supplementary Table S4. Spearman correlation matrix opposing volumetric rates of N₂ fixation and primary production (PP) to depth-specific environmental variables for the combined Belgica 2014/14 and GEOVIDE cruises. The correlation factor (r) and its significance given by the p-value (*p*) at p < 0.001, p < 0.01 and p < 0.05 presented with ***, ** and *, respectively, and the number of observations (n) are shown for each combination tested. Note that when nutrient concentrations were < DL we used the DL value to run the correlation test. Also, P* correlations were only made for the Belgica 2014/14 studied sites and dFe correlations only for GEOVIDE sampling stations.

		Salinity	$[{ m NH_4}^+]$ [μM]	$[NO_{3}^{-} + NO_{2}^{-}]$ [μ M]	In situ P* [µM]	DFe [nM] (Tonnard et al., 2018)	Chl a [µg L^{-1}]	РР [µmol C m ⁻³ d ⁻¹]	N ₂ fixation [nmol N L ⁻¹ d ⁻¹]
Temperature	r	-0.191	-0.418**	-0.854***	0.628**	0.291	0.00461	0.158	0.54***
[°C]	р	0.202	0.00398	0.0000002	0.00104	0.267	0.976	0.292	0.00012
	n	46	46	46	24	16	43	46	46
Salinity			0.0922	0.39**	0.0288	0.0883	-0.195	-0.214	0.106
			0.54	0.00765	0.892	0.738	0.208	0.152	0.482
			46	46	24	16	43	46	46
$[\mathrm{NH_4}^+]$				0.344*	-0.441*	-0.462	0.128	0.255	-0.154
[µM]				0.0194	0.031	0.0695	0.411	0.0869	0.307
				46	24	16	43	46	46
$[NO_3^- + NO_2^-]$					-0.716***	-0.253	-0.216	-0.314*	-0.362*
[µM]					0.0000325	0.336	0.162	0.0338	0.0136
					24	16	43	46	46
In situ P*						undefined	0.149	0.369	0.557**
[µM]						undefined	0.481	0.0746	0.00481
						0	24	24	24
DFe							-0.57	-0.416	0.175
[nM]							0.0322	0.104	0.505
(Tonnard et al., 2018)							14	16	16
Chl a								0.85***	-0.0874
[µg L ⁻¹]								0.0000002	0.575
								43	43
PP									0.143
$[\mu mol C m^{-3} d^{-1}]$									0.342
									46



Supplementary Figure S2. High resolution trihourly averaged sea surface temperature (SST) on May 29th 2014 (sampling date of station Bel-13, 10 days following the sampling of station Geo-1), derived from the European Sea (sea surface subskin temperature) of Copernicus Marine Environment Monitoring Service (CMEMS, European Commission). White markers indicate the location of the stations sampled during our study (image provided by Google Earth Pro).



Supplementary Figure S3. April (A) and May (B) 2014 monthly averaged dry + wet dust deposition (kg $m^{-2} s^{-1}$) derived from Giovanni online satellite data system (NASA Goddard Earth Sciences Data and Information Services Center). White markers indicate the location of the stations sampled during our study (image provided by Google Earth Pro).