Supplementary Material

Evidence of high N_2 fixation rates in productive waters of the temperate Northeast Atlantic

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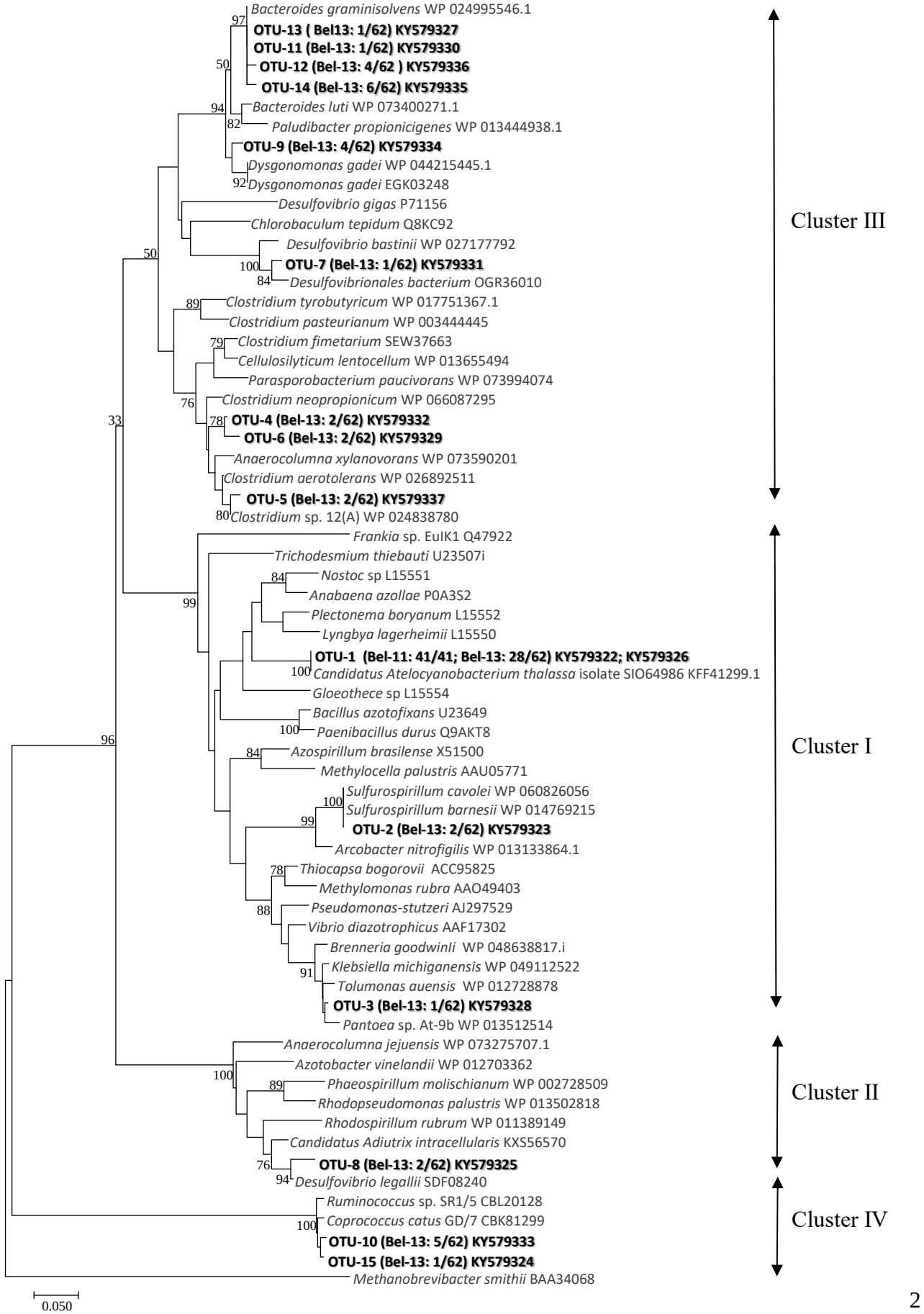
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OTU-14 (Bel-13: 6/62) KY579335
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Paludibacter propionicigenes WP 013444938.1
OTU-9 (Bel-13: 4/62) KY579334
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Chlorobaculum tepidum Q8KC92
Desulfovibrio bastinii WP 027177792
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Clostridium pasteurianum WP 003444445
Clostridium fimetarium SEW37663
Cellulosilyticum lentocellum WP 013655494
Parasporobacterium paucivorans WP 073994074
Clostridium neopropionicum WP 066087295
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Anaerocolumna xylanovorans WP 073590201
Clostridium aerotolerans WP 026892511
OTU-5 (Bel-13: 2/62) KY579337
Clostridium sp. 12(A) WP 024838780
Frankia sp. EuIK1 Q47922
Trichodesmium thiebauti U23507i
Nostoc sp L15551
Anabaena azollae P0A3S2
Plectonema boryanum L15552
Lyngbya lagerheimii L15550
OTU-1 (Bel-11: 41/41; Bel-13: 28/62) KY579322; KY579326
Candidatus Atelocyanobacterium thalassa isolate SIO64986 KFF41299.1
Gloeothece sp L15554
Bacillus azotofixans U23649
Paenibacillus durus Q9AKT8
Azospirillum brasilense X51500
Methylocella palustris AAU05771
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Pseudomonas-stutzeri AJ297529
Vibrio diazotrophicus AAF17302
Brenneria goodwinIi WP 048638817.i
Klebsiella michiganensis WP 049112522
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Pantoea sp. At-9b WP 013512514
Anaerocolumna jejuensis WP 073275707.1
Azotobacter vinelandii WP 012703362
Phaeospirillum molischianum WP 002728509
Rhodopseudomonas palustris WP 013502818
Rhodospirillum rubrum WP 011389149
Candidatus Adiutrix intracellularis KXS56570
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Desulfovibrio legallii SDF08240
Ruminococcus sp. SR1/5 CBL20128
Coprococcus catus GD/7 CBK81299
OTU-10 (Bel-13: 5/62) KY579333
OTU-15 (Bel-13: 1/62) KY579324
Methanobrevibacter smithii BAA34068
0.050
Cluster III
Cluster I
Cluster II
Cluster IV

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 (≥ 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 (≥ 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]	NH_4^+ [µM]	NO_3^-+NO_2^- [µM]	P* [µM]	Chl *a* [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	**< DL**	**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]	NH_4^+ [µM]	NO_3^-+NO_2^- [µM]	P* [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]	NH_4^+ [µM]	NO_3^-+NO_2^- [µM]	P* [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.

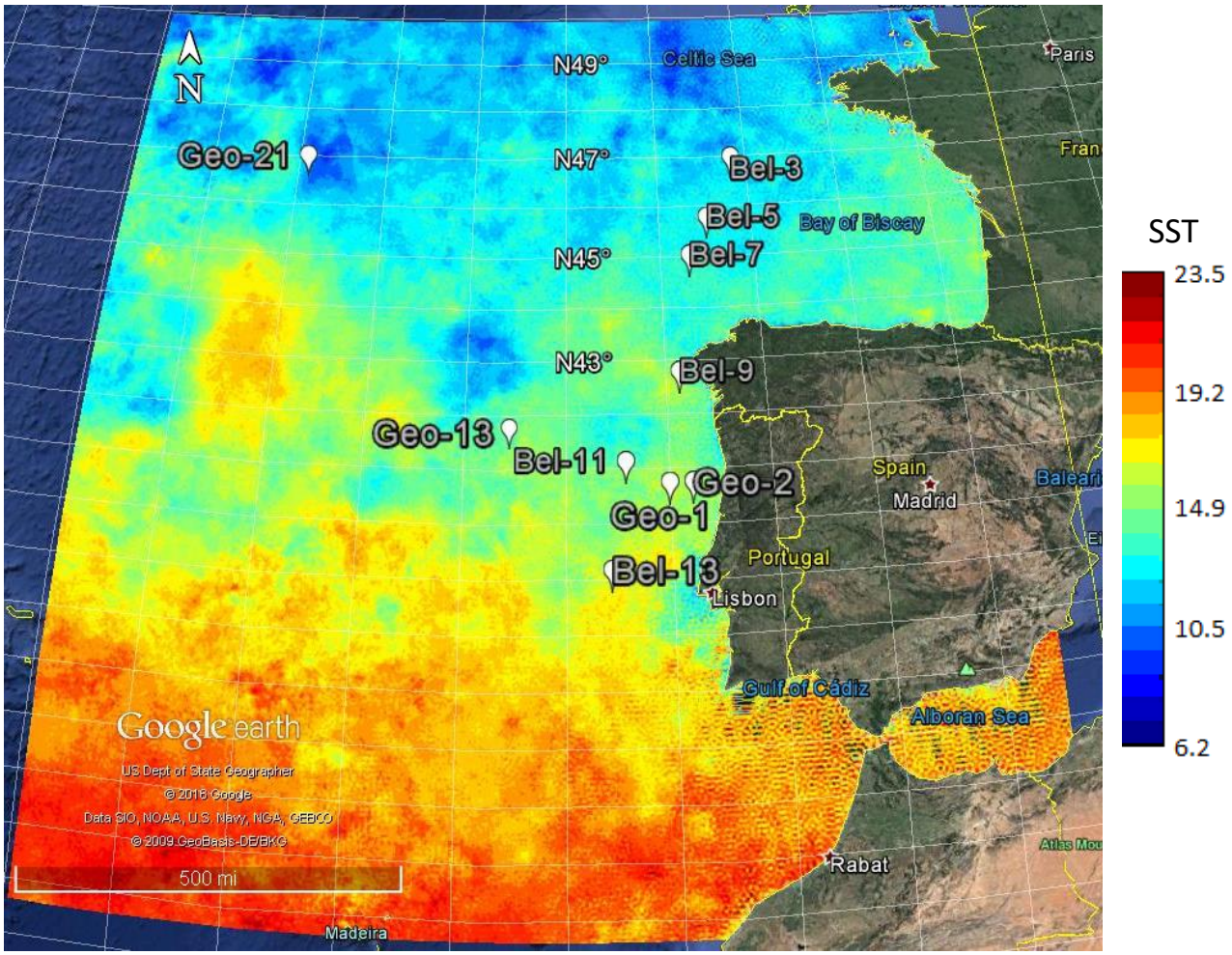
Station	MLD	Lat. [°N]	Long. [°E]	**N_2 fixation [µmol N m^{-2} d^{-1}]**	**Primary Production [mmol C m^{-2} d^{-1}]**	Euphotic layer averaged Temperature [°C]	Euphotic layer averaged Salinity [psu]	Euphotic layer integrated Chl *a* [mg m^{-2}]	Euphotic layer integrated NH_4^+ [µmol m^{-2}]	Euphotic 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 N_2 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.

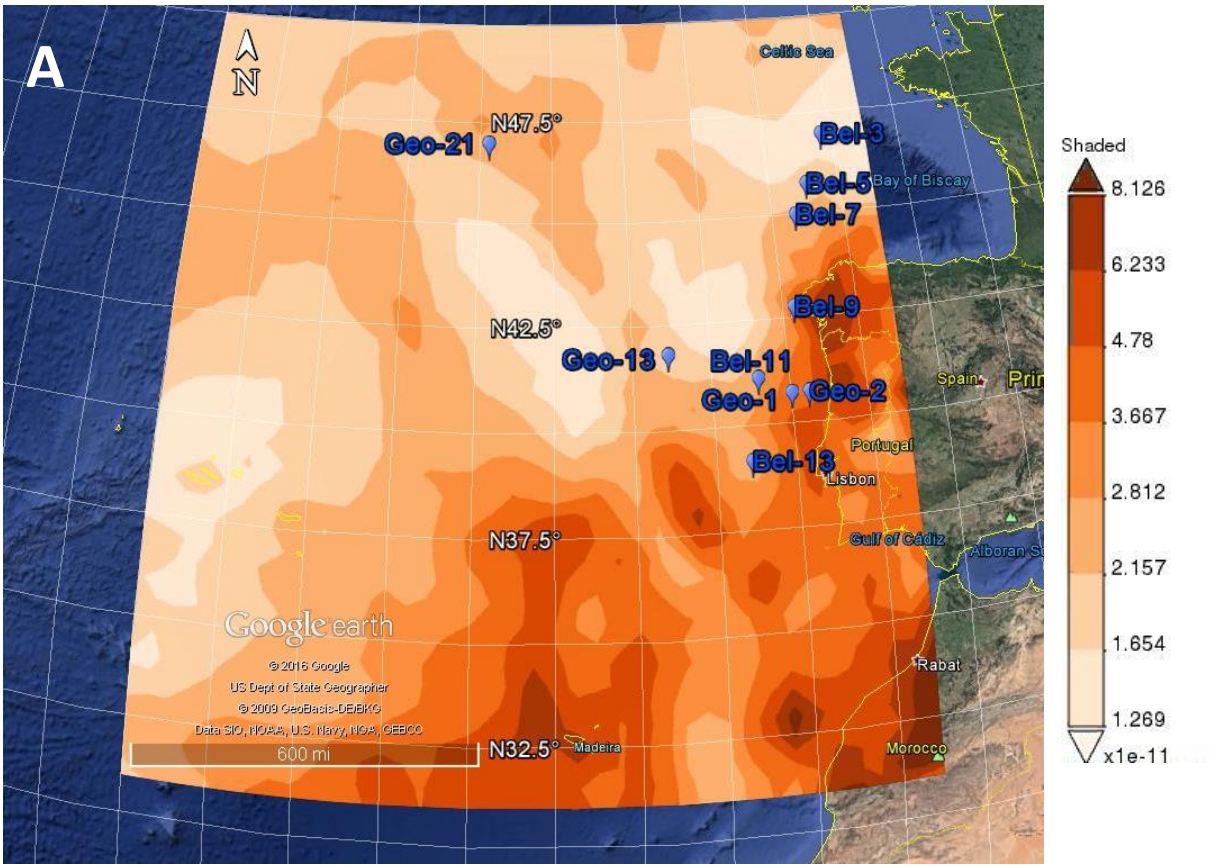
		Average salinity	Integrated $[NH_4^+]$ [µmol m^{-2}]	Integrated $[NO_3^- + NO_2^-]$ [µmol m^{-2}]	Average *in situ* P* [µM]	Surface climatology P* [µM]	Integrated DFe [nmol m^{-2}]	Dust deposition April 2014 (kg m^{-2} s^{-1})	Dust deposition May 2014 (kg m^{-2} s^{-1})	**PP [mmol m^{-2} d^{-1}]**	**N_2 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	*p*	*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**
$[NH_4^+]$				*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*
(kg m^{-2} s^{-1})									10	10	10
Dust deposition										**0.512**	**-0.774** **
May 2014										*0.116*	*0.00686*
(kg m^{-2} s^{-1})										10	10
PP											**-0.0365**
[mmol C m^{-2} d^{-1}]											*0.892*
											10

Supplementary Table S4. Spearman correlation matrix opposing volumetric rates of N_2 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	$[NH_4^+]$ [µM]	$[NO_3^- + NO_2^-]$ [µM]	*In situ* P* [µM]	DFe [nM] (Tonnard et al., 2018)	Chl *a* [µg L^{-1}]	**PP [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]	*p*	*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
$[NH_4^+]$				**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**
[µmol 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).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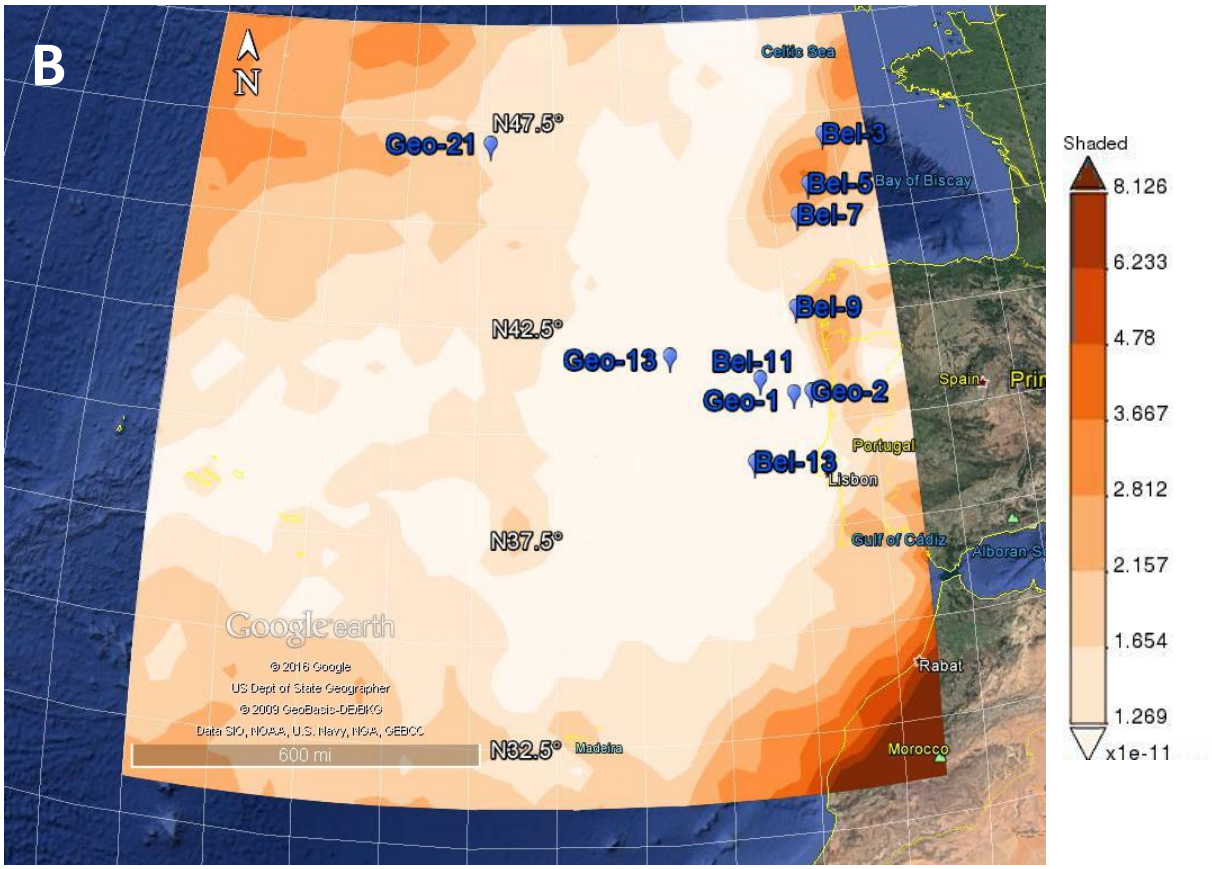
x1e-11


B
N
Celtic Sea
N47.5°
Geo-21
Bel-3
Bel-5
Bay of Biscay
Bel-7
Bel-9
N42.5°
Geo-13
Bel-11
Geo-1
Geo-2
Spain
Portugal
Bel-13
Lisbon
N37.5°
Gulf of Cádiz
Google earth
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US Dept of State Geographer
© 2009 GeoBasis-DE/BKG
Data SIO, NOAA, U.S. Navy, NGA, GEBCO
600 mi
N32.5°
Madeira
Rabat
Morocco
Shaded
8.126
6.233
4.78
3.667
2.812
2.157
1.654
1.269
x1e-11

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).