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Supporting Information for

[Nitrogen fixation in the eastern Atlantic reaches similar levels in the southern and northern hemisphere]

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Introduction

[During the ANT XXIX/1 EUROPA cruise in the eastern Atlantic Ocean (38°N to 21°S; Nov. 2012) we investigated euphotic layer dinitrogen (N_2) fixation and primary production (PP). Samples dedicated combined tracer ($^5N_2$ and ^{13}C-bicarbonate) incubation experiments were collected at depths equivalent to 50, 15, 3.5 and 0.5% of surface irradiance. N_2 fixation was measured using the $^{15}N_2$ dissolution incubation method [*Mohr et al.,* 2010; *Großkopf et al.,* 2012].

Supporting material presented here includes:

- **Text S1**, extended description of the methodology
    - For $^{15}N_2$ incubation experiments: checking the 98% enriched $^{15}N_2$ gas brand for potential contamination with ^{15}N-labelled substrates other than N_2, and checking the risk of incubation fertilization with nutrients or trace elements due to the use of natural Sargasso Sea surface water as matrix to prepare $^{15}N_2$-enriched seawater.
    - For the $^{15}NO_3^-$ incubation: protocol and selection of data used in the discussion

- **Two sets of figures** used as circumstantial evidence illustrating
    - the combined effect of the Benguela upwelling system and the Benguela and Angola Currents on offshore nutrient climatology and chlorophyll satellite data at the time of the current study;
    - the atmospheric dust deposition in the eastern Atlantic Ocean during the sampling month of this study

- **Table S1** illustrating the collection of published N_2 fixation rates in the North and South Atlantic Ocean used in the discussion to compare our measurements to earlier works; and also used to compute annual N input through N_2 fixation in the Atlantic

- **Table S2**: Summary dataset used to run principal component analysis relating N_2 fixation measurements to phosphorus excess and dust input.

- **One dataset** presenting the complete set of field measurements during the ANT XXIX/1 cruise: sampling sites, nutrients concentrations (nitrate+nitrite, phosphate and ammonium), rate measurements of N_2 fixation, primary production and nitrate uptake rates.]

Text S1.

Incubations experiments

Potential biases related to $^{15}N_2$ incubation

There are some biases that may arise from the $^{15}N_2$ incubation experiments protocol [*Dabundo et al.,* 2014; *Klawonn et al.,* 2015] among which: (i) a contamination of the $^{15}N_2$ spike gas with ^{15}N-labeled bioavailable nitrogen (N) contaminants [*Dabundo et al.,* 2014]; and (ii) the low nutrient seawater (natural Atlantic seawater, OSIL) containing the $^{15}N_2$ spike gas and potentially having a different chemical composition than ambient seawater (nutrients, trace metals, stoichiometry).

(i) A recent study revealed that some brands of commercial $^{15}N_2$ gas potentially contained ^{15}N-labeled bioavailable N contaminants (nitrate+nitrite, NO_3^- + NO_2^-, ammonium, NH_4^+ and nitrous oxide, N_2O) which caused an overestimation or false detection of N_2 fixation rates [*Dabundo et al.,* 2014]. Therefore the used $^{15}N_2$ gas (Eurisotop, 98%+, lot number 23/051301) was tested in our laboratory for contamination with $^{15}NO_3^-$, $^{15}NO_2^-$ and $^{15}NH_4^+$. A first batch was prepared to check for any contamination with $^{15}NO_3^-$ and $^{15}NO_3^-$ + $^{15}NO_2^-$. Potential ^{15}N isotopic enrichment in these substrates was verified using the denitrifier method described by *Sigman et al.* [2001] for solutions previously treated or not with sulfamic acid to retrieve all traces of NO_2^- [*Granger and Sigman,* 2009]. The second batch aimed at checking for contamination with $^{15}NH_4^+$+$^{15}NO_2^-$. Here we applied the azide method described by *McIlvin and Altabet* [2005] with a hypobromite pre-treatment as presented by *Zhang et al.* [2007] in order to convert all NH_4^+ in to NO_2^-. For each test we investigated the impact of $^{15}N_2$ gas injection (1 mL) on the N isotopic composition of ~5 µmol L^{-1} of international NO_3^- and NH_4^+ reference solutions (IAEA-N3; NO_3^-; $\delta^{15}N$ of 4.72‰ and IAEA-N2; NH_4^+; $\delta^{15}N$ of 20.41‰) prepared in degassed low nutrient OSIL seawater (60 mL per treatment). The N isotopic signature of triplicate preparations for each test experiment were compared to signals of control standards also prepared in low nutrient seawater but not injected with $^{15}N_2$ gas. Student's paired t-test between average controls and treatments for both methods returned consistently no significant difference: for the sulfamic/denitrifier method (p-value = 0.40; control treatments $\delta^{15}N$ of (NO_3^- + NO_2^-) of 4.81 ± 0.03‰, n = 3, versus labeled treatments $\delta^{15}N$ of (NO_3^- + NO_2^-) of 4.75 ± 0.12‰, n = 9), for the denitrifier method only (p-value = 0.08; control treatments $\delta^{15}N$-NO_3^- of 4.72 ± 0.11‰, n = 5 versus labeled treatments $\delta^{15}N$-NO_3^- of 4.87 ± 0.18‰, n = 15) and for the hypobromite/azide method (p-value = 0.09; controls treatments $\delta^{15}N$ of (NH_4^+ + NO_2^-) of 20.30 ± 0.23‰, n = 5

versus labeled treatments $\delta^{15}N$ of (NH_4^+ + NO_2^-) of 20.10 ± 0.21‰, n = 15). We therefore consider our labeled gas source was free of contamination of ^{15}N labeled NO_3^-, NO_2^- or NH_4^+.

(ii) A second potential bias may result from the addition of $^{15}N_2$ amended 250 mL of natural Sargasso Sea surface water (OSIL) to 4250 mL of sampled seawater since this could potentially alter the chemical properties of the original waters (i.e., nutrients and trace metals) [Klawonn et al., 2015], affecting in turn N_2 fixation rates. The Sargasso Sea water is collected in an area between 35 to 40°N and 42 to 49°W (personal communication from the supplier, OSIL, UK) and has an average salinity of 35. In order to test for any contamination with this water we analyzed four different batches for nutrients (NH_4^+, NO_3^-+NO_2^-, and PO_4^{3-}) and trace metals (iron, Fe and molybdenum, Mo). Fe and Mo are essential co-factors in the N_2-fixing enzyme complex "nitrogenase" [*Raven,* 1988]. NH_4^+ and PO_4^{3-} concentrations were analyzed using standard fluorometric [Holmes et al., 1999] and colorimetric methods [Grasshoff et al., 1983], respectively. NO_3^-+NO_2^- concentrations were measured by chemiluminescence detection of nitric oxide (NO) [*Braman and Hendrix,* 1989] using a Teledyne T200 chemiluminescence NOx analyzer (Thousand Oaks), following a reduction step to NO in a hot (95°C) acidic vanadium (III) solution. Dissolved Fe (dFe) and Mo (dMo) concentrations were measured using the seaFAST S2 / pico (Elemental Scientific Inc., ESI) with online solid-phase extraction onto Nobias chelate PA-1 resin [*Lagerström et al.,* 2013; *Quéroué et al.,* 2014] and Sector Field-Inductively Coupled Plasma-Mass Spectrometry (XR-ICP-MS, Thermo Element). Taking into account the dilution factor (i.e., 250/4580 = 0.055), the addition of the OSIL seawater into the incubation bottles increased the concentration by < 16, < 6, < 6, < 0.2, and < 11 nmol L^{-1} for NH_4^+, NO_3^-+NO_2^-, PO_4^{3-}, dFe, and dMo, respectively, thus keeping the concentrations in the incubation bottles within the range of observed values in the open North and South Atlantic waters: < 50 to 500 nmol L^{-1} for NH_4^+, < 10 to 300 nmol L^{-1} for NO_3^- [*Rees et al.,* 2006], < 10 to 300 nmol L^{-1} for PO_4^{3-} [*Schlosser et al.,* 2014; *Snow et al.,* 2015], 0.1 to 1.1 nmol L^{-1} for dFe and 80 to 140 nmol L^{-1} for dMo [*Sarthou et al.,* 2003; *Mawji et al.,* 2015; *Pinedo-González et al.,* 2015; *Von Der Heyden and Roychoudhury,* 2015]. We did not analyze the OSIL water for DON content. However DON concentration in surface waters of the global ocean has been reported to exhibit a very narrow range of concentrations from 2 to 7 µmol kg^{-1} [*Letscher et al.,* 2013]. North Atlantic surface waters shows an east-to-west gradient in DON concentration from ~ 4.5 µmol kg^{-1} in the west (Sargasso Sea) to > 5 µmol kg^{-1} in the east [*Mahaffey et al.,* 2004; *Roussenov et al.,* 2006; *Charria et al.,* 2008; *Torres-Valdés et al.,* 2009; *Letscher et al.,* 2013]. Thus it is unlikely that the Sargasso

seawater used to amend the $^{15}N_2$ incubations would have added extra DON to the natural samples.

To conclude, although we acknowledge that using *in situ* surface seawater for preparing the $^{15}N_2$ spike is more rigorous to assess N_2 fixation, the contaminations with both the $^{15}N_2$ gas and the addition of Sargasso Sea surface water can be considered as negligible for this study.

Nitrate uptake data

During the ANT XXIX/1 expedition NO_3^- uptake rate was determined at the same 4 PAR levels as the N_2 fixation uptake experiments. Per depth, duplicate (initial + final) 1 L acid-cleaned polycarbonate bottles were filled with sample water, spiked with 1 mL of a 22 µM $Na^{15}NO_3$ (98%, Sigma-Aldrich) at stations 9 to 13 and 1 mL of a 1 mM, $Na^{15}NO_3$ for the remaining stations. These additions yielded initial $^{15}N\%$-NO_3^- of 0.5–95% (37 ± 5%, n = 60). After 24 h incubation bottles were filtered for POC/PN concentration and isotopic signature assessment. POC/PN measurements were performed on an Elemental Analyzer (Flash EA112) coupled to an isotope ratio mass spectrometer (IRMS, Delta V, Thermo) via an Elemental Analyzer, EA-IRMS). Rates were computed by measuring isotopic tracer enrichments in the organic matter at termination of the incubations according to *Dugdale and Wilkerson* [1986].

Note that for stations 1 to 8 and 14, $^{15}NO_3^-$ tracer additions were >> 10% of ambient NO_3^- concentration [*Dugdale and Goering,* 1967] thereby causing a significant fertilization effect. As a result NO_3^- uptake rates obtained for those stations were not considered to compute N_2 fixation contribution to new production.

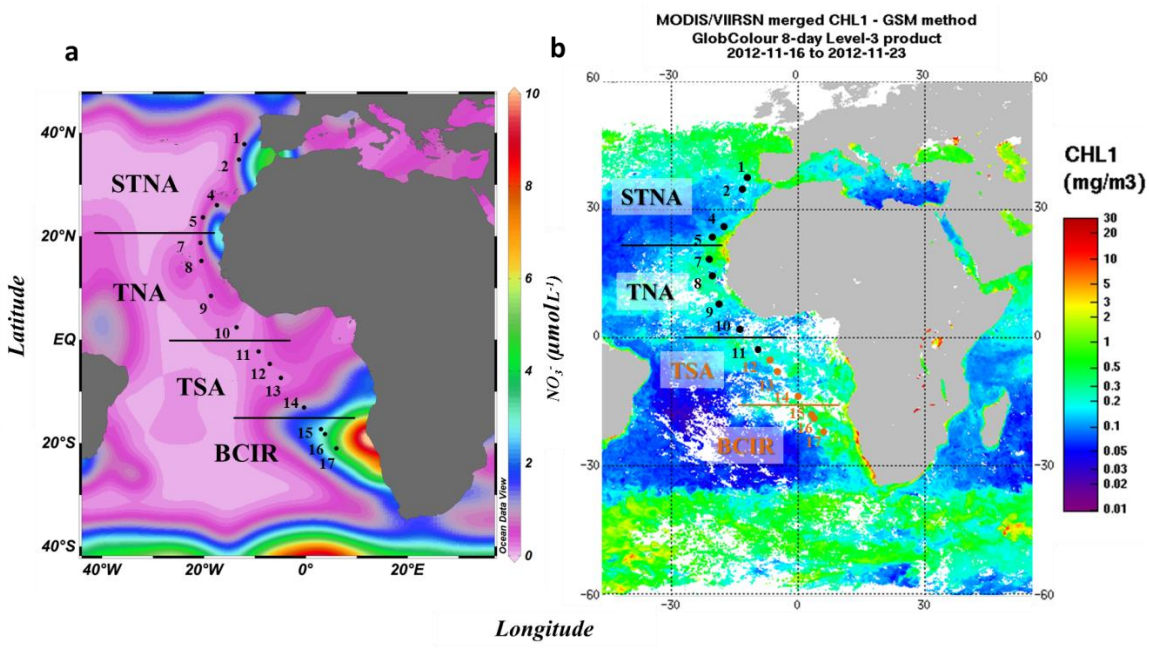

Figure S1. (a) November, monthly average nitrate concentration from 1955 to 2012 at 20 m depth (World Ocean Atlas 2013) [Garcia et al., 2013]. Dots indicate the locations of the stations sampled during the ANT XXIX/1 cruise (b) Chlorophyll recorded by the MODIS satellite from November 16th to 23rd 2012, corresponding to the sampling period of stations 12 to 17 (in orange dots) [based on Maritorena and Siegel, 2005].

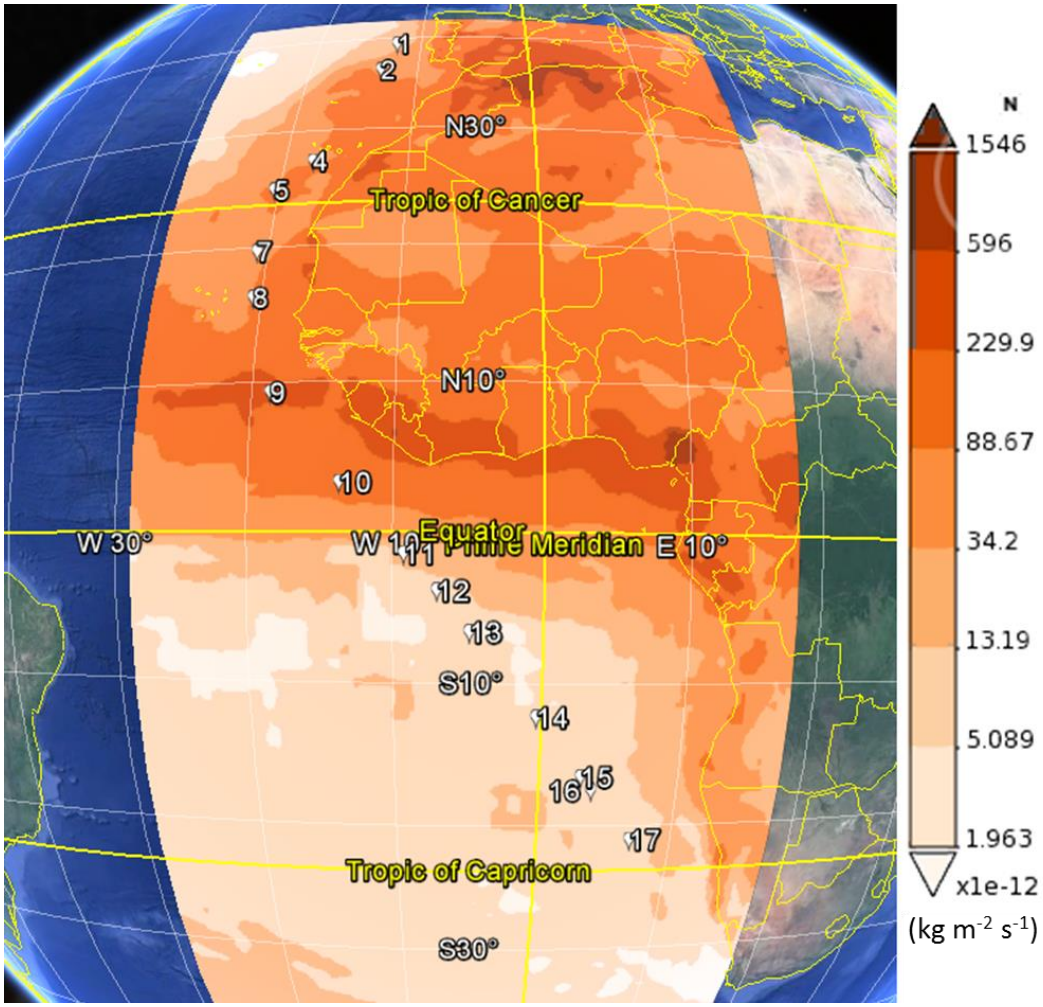

Figure S2. Monthly averaged combined dry + wet dust deposition (kg m-2 s-1) derived from Giovanni online satellite data system (NASA Goddard Earth Sciences Data and Information Services Center). White markers indicate the location of the stations sampled during our study.

Table S1. : N_2 fixation rates in the North and South Atlantic; Overview of published data (large table in excel file).

Table S2. Summary dataset used to run principal component analysis relating N_2 fixation measurements to phosphorus excess and dust input.

Station	Latitude [°N]	Longitude [°W]	Surface N_2 fixation ($\mu mol\ m^{-3}\ d^{-1}$)	Euphotic layer-integrated N_2 fixation ($\mu mol\ m^{-2}\ d^{-1}$)	Average P* 100-700 m ($\mu mol\ L^{-1}$)	Dry + Wet Dust deposition ($\mu g\ m^{-2}\ d^{-1}$)
1	37.83	-12.09	2.71	140.6	-0.02	5308.0
2	34.88	-13.14	0.68	59.3	0.02	13762.2
4	26.05	-17.46	2.42	124.8	-0.04	13762.2
5	23.69	-20.18	3.98	141.3	-0.30	13762.2
7	18.76	-20.70	12.50	370.2	-0.03	5308.0
8	15.25	-20.52	NA	NA	0.22	13762.2
9	8.47	-18.62	1.94	109.2	0.35	35678.9
10	2.41	-13.60	0.39	79.4	0.20	13762.2
11	-2.26	-9.25	1.99	118.8	0.19	2047.2
12	-4.67	-7.06	0.84	53.5	0.78	789.7
13	-7.39	-4.90	0.76	46.9	0.15	789.7
14	-13.10	-0.33	0.81	80.1	0.55	789.7
15	-17.28	2.98	1.95	93.2	0.71	789.7
16	-18.25	3.78	0.76	72.7	0.35	789.7
17	-20.99	6.00	1.29	51.5	0.14	789.7

Data Set S1. Complete set of field measurements during the ANT XXIX/1 cruise.

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