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BRIEF COMMUNICATION

Quantifying the overestimation of planktonic N_2 fixation due to contamination of $^{15}N_2$ gas stocks

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The $^{15}N_2$-tracer assay [Montoya *et al.* (1996) A simple, high-precision, high-sensitivity tracer assay for N_2 fixation. *Appl. Environ. Microbiol.*, **62**, 986–993.] is the most used method for measuring biological N_2 fixation in terrestrial and aquatic environments. The reliability of this technique depends on the purity of the commercial $^{15}N_2$ gas stocks used. However, Dabundo *et al.* [(2014) The contamination of commercial $^{15}N_2$ gas stocks with ^{15}N-labeled nitrate and 142 ammonium and consequences for nitrogen fixation measurements. *PLoS One*, **9**, e110335.] reported the contamination of some of these stocks with labile ^{15}N-labeled compounds (ammonium, nitrate and/or nitrite). Considering that the tracer assay relies on the conversion of isotopically labeled $^{15}N_2$ into organic nitrogen, this contamination may have led to overestimated N_2 fixation rates. We conducted laboratory and field experiments in order to (i) test the susceptibility of ^{15}N contaminants to assimilation by non-diazotroph organisms and (ii) determine the potential overestimation of the N_2 fixation rates estimated in the field. Our findings indicate that the contaminant ^{15}N-compounds are assimilated by non-diazotrophs organisms, leading to an overestimation of N_2 fixation rates in the field up to 16-fold under hydrographic conditions of winter mixing.

KEYWORDS: marine N_2 fixation; $^{15}N_2$ contamination; nitrogen isotopes; diazotrophs

Biological N_2 fixation is an important source of nitrogen into the ocean (Gruber, 2008), and the $^{15}N_2$-tracer addition technique (Montoya *et al.*, 1996) is the most extended method for measuring it in both terrestrial and aquatic environments. The $^{15}N_2$-tracer assay is based on the assimilation and transformation of isotopically

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labeled $^{15}N_2$ into organic nitrogen in incubations of natural planktonic communities. The accuracy of this approach is based on the assumption that the ^{15}N enrichment of particulate matter can only be due to biological reduction of N_2. Nevertheless, Dabundo *et al.* (2014) reported the presence of substantial concentrations of ^{15}N-contaminants ($^{15}NH_4^+$, $^{15}NO_3^-$, $^{15}NO_2^-$) in some commercial $^{15}N_2$ gas stocks supplied by Sigma-Aldrich, which may be assimilated by microorganisms. Their findings imply that the contaminant ^{15}N-compounds could lead to significant overestimations in some of the N_2 fixation rates reported in the literature. However, the magnitude of the overestimation of N_2 fixation measured in the field when using contaminated $^{15}N_2$ remains unknown.

In the framework of the NICANOR project, biological N_2 fixation was quantified between 2014 and 2015 in a station located in productive waters of the temperate upwelling region off NW Iberian Peninsula (off Ría de A Coruña, 43.42°N–8.44°W; depth = 80 m) (see Supplementary data S1 and Moreira-Coello *et al.*, 2017). Most of N_2 fixation rates measured throughout 2014 reached values comparable to the higher rates described for (sub) tropical oligotrophic regions (up to 82 nmol N L^{-1} d^{-1}) (Luo *et al.*, 2012). The concern that this could be due to the use of a $^{15}N_2$ gas stock substantially contaminated with ^{15}N-labeled ammonium, nitrate and nitrite motivated further laboratory and field experiments. The main goals of this study were (i) to test the susceptibility of ^{15}N-contaminants to assimilation by non-diazotroph organisms and (ii) to determine the magnitude of potential overestimation of the biological N_2 fixation rates estimated in the field.

In order to investigate the potential assimilation of ^{15}N-contaminants by non-diazotrophic organisms, the marine green alga *Tetraselmis suecica* was cultured in f/2 growth media (f/32 for nitrate) equilibrated with $^{15}N_2$ from different commercial suppliers: Sigma-Aldrich lot MBBB0968V and Cambridge Isotope lot I-19168A. The Sigma lot showed substantial contamination with ^{15}N-labeled ammonium, nitrate and nitrite (Dabundo *et al.*, 2014), whereas the composition of the specific Cambridge lot used was unknown. However, other analyzed stocks of the same commercial supplier showed negligible contamination (Dabundo *et al.*, 2014). Three 250-mL Erlenmeyer flasks were filled with 200 mL of growth medium equilibrated during 60 h with $^{15}N_2$ from the Sigma stock, three with medium equilibrated with $^{15}N_2$ from the Cambridge stock and finally another three with unamended medium (control). The nine flasks were inoculated with a stock culture of *T. suecica* (28 μg L^{-1} of initial concentration of chlorophyll *a* in each flask) and incubated during 4 days. For comparing treatments we used the $\delta^{15}N$ parameter, which expresses the $^{15}N/^{14}N$ isotopic ratio in a sample in relation to the standard value in the atmospheric N_2. The treatment with the Sigma stock resulted in statistically significant (Kruskal–Wallis, $P < 0.001$) enrichments in ^{15}N abundance of the particulate organic nitrogen ($\delta^{15}N_{PN} \sim 1.5‰$), in relation to the treatment with the Cambridge stock and the control ($\delta^{15}N_{PN}$ ranging from -0.90 ± 0.01 to $-1.4 \pm 0.3‰$), both during the exponential growth (Day 2) and stationary phase under nitrate-depleted conditions (Day 4). Sigma cultures were ^{15}N enriched because the microalgae assimilated the inorganic ^{15}N compounds present in the contaminated lecture bottle. No statistically significant (Kruskal–Wallis, $P > 0.05$) differences were observed between the control and the cultures equilibrated with $^{15}N_2$ from the Cambridge stock (Fig. 1). If we compute the N_2 fixation rate in the cultures of *T. suecica* equilibrated with contaminated $^{15}N_2$ from Sigma assuming that this microalga is a N_2-fixer, the observed difference in $\delta^{15}N$ and ^{15}N atom% between control and Sigma treatments would result in an apparent N_2 fixation rate ranging from 0.6 to 1.1 nmol N L^{-1} d^{-1}, after 4 and 2 days of incubation, respectively. This value of N_2 fixation is an artifact since *T. suecica* is not a diazotroph. Therefore, in a system where N_2 fixation is *per se* relatively low, the effect of using contaminated $^{15}N_2$ may be significant. More details on this experiment are available in Supplementary data S2.

In December 2014, we measured biological N_2 fixation rates in the field at surface waters and at 70-m depth by the $^{15}N_2$-uptake technique (Montoya *et al.*, 1996) using concomitantly two $^{15}N_2$ lecture bottles, the contaminated

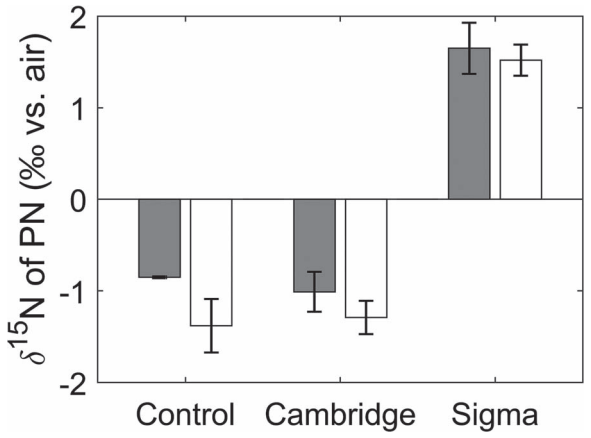

Fig. 1. ^{15}N content of particulate organic nitrogen versus the reference value in atmospheric N^2 ($\delta^{15}N_{PN}$, ‰ vs. air) in *T. suecica* cultures equilibrated with $^{15}N_2$ gas from Cambridge Isotope and Sigma-Aldrich commercial stocks sampled 2 days (exponential growth, gray bars) and 4 days (stationary phase under nitrate-depleted conditions, white bars) after inoculation. Error bars represent standard deviation (n=3).

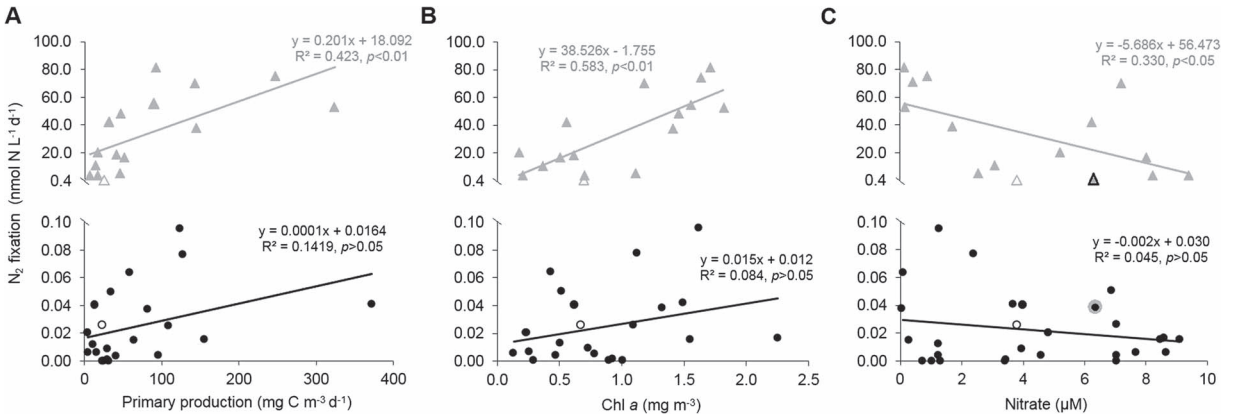

Fig. 2. (**A**) Total primary production, (**B**) chlorophyll *a* concentration and (**C**) nitrate concentration versus volumetric biological N_2 fixation rates obtained in the framework of the NICANOR project (February 2014 to December 2015) by using the contaminated Sigma-Aldrich lot (gray triangles), and the presumably non-contaminated Cambridge Isotope lot (black circles). Samples in which N_2 fixation was measured using both Sigma and Cambridge stocks are represented for December 2014 at 0 m depth by empty triangles and empty circles, respectively, and for December 2014 at 70 m by a gray triangle with black edge and a black circle with gray edge. Regression lines are plotted for each data set. Only samples with chlorophyll *a* < 2.5 mg m^{-3} have been represented in order to improve the data visualization in panels A and B, leaving out a sample with the highest chlorophyll *a* (6.5 mg m^{-3}) and primary production (396.4 mg C m^{-3} d^{-1}) and N_2 fixation of ~72 nmol N L^{-1} d^{-1} obtained using the contaminated Sigma stock.

gas from Sigma (stock MBBB0968V) and the presumably non-contaminated gas from Cambridge (I-19168A), and compared the results to ascertain the potential overestimation of N_2 fixation rates caused by the assimilation of ^{15}N contaminants. This experiment is described in detail in Supplementary data S3. These results were obtained under hydrographic conditions of winter mixing, which was characterized by a deep mixed layer (51 m), low chlorophyll *a* concentration (0.2–0.7 mg m^{-3}) and low primary production (4.8–23.5 mg C m^{-3} d^{-1}) (Fig. S2). The volumetric rates of N_2 fixation obtained were significantly higher, by a factor of 16, when using $^{15}N_2$ from Sigma (0.4 ± 0.1 and 0.6 ± 0.3 nmol N L^{-1} d^{-1}, respectively) than when using the Cambridge stock (0.03 ± 0.01 and 0.04 ± 0.01 nmol N L^{-1} d^{-1}, respectively) (Kruskal–Wallis, $P < 0.05$) (calculations are provided in Supplementary data S4 and S5). The Cambridge gas stock showed undetectable contamination according to our results with *T. suecica* cultures (Fig. 1), and the N_2 fixation rates equal to zero, or below the detection limit, measured in our field experiments during 2015 (Fig. 2). The reliable rates obtained by using the Cambridge stock in the samplings of 2015 and December 2014 did not correlate significantly either with total primary production ($R^2 = 0.142$, $P > 0.05$), chlorophyll *a* ($R^2 = 0.084$, $P > 0.05$) or environmental nitrate concentration ($R^2 = 0.045$, $P > 0.05$). The N_2 fixation measured during 2014 using the contaminated Sigma stock, including the December sampling, ranged from 0.4 to 82 nmol N L^{-1} d^{-1} and correlated positively with both primary production ($R^2 = 0.423$, $P < 0.01$) and chlorophyll *a* ($R^2 = 0.583$, $P < 0.01$), and negatively with nitrate concentration ($R^2 = 0.330$, $P < 0.05$) (Fig. 2). Thus, lower overestimation of N_2 fixation rates occurred in conditions of elevated nitrate concentration. By contrast, higher overestimation of N_2 fixation rates occurred in conditions of high productivity and biomass. The high phytoplankton production and biomass characteristic of conditions such as spring or summer blooms are linked to a high assimilation of nutrients, among which are the contaminants included in the stocks. Hence, the magnitude of overestimation depends on biomass and productivity of the phytoplankton present. In this coastal region, both variables are subject to marked seasonal variability and influenced by wind-driven upwelling, causing large changes in phytoplankton dynamics over short temporal scales (Gilcoto *et al.*, 2017). Thus, the overestimation will be different according to the prevailing hydrographic setting. In the widely studied oligotrophic (sub)tropical oceans, where N_2 fixers predominate, inflated rates may have gone unnoticed due to the high N_2 fixation rates that commonly characterize these systems.

The overestimated N_2 fixation rates due to the contamination obtained during 2014 ranged from 310 to 2260 µmol N m^{-2} d^{-1} (0.4–82 nmol N L^{-1} d^{-1}, Fig. 2), reaching similar values to the highest ever reported in (sub)tropical regions (Luo *et al.*, 2012). These rates would imply a depth-integrated diazotrophic biomass between 80 and 600 mg C m^{-2}, representing between 4% and 25% of the total phytoplankton biomass in the station

studied (Teira *et al.*, 2003), which is highly unrealistic (details on these calculations available in Supplementary data S6).

The experiment with *T. suecica* confirmed the uptake of ^{15}N contaminants by non-diazotrophs. Furthermore, the comparison, through field estimates, of the N_2 fixation rates obtained simultaneously with the Sigma and Cambridge stocks allowed to demonstrate that using contaminated $^{15}N_2$ yielded an overestimation of the rates by up to 16-fold. It is expected that the assimilation of dissolved inorganic nitrogen, mainly ammonium and nitrate, is preferred to the energy-costly N_2 fixation process (Stam *et al.*, 1987). Thus, the estimates of N_2 fixation reported in the literature may be distorted depending on the commercial $^{15}N_2$ source used. Past reports obtained using $^{15}N_2$ gas from Sigma should be interpreted with caution. However, the published rates obtained with $^{15}N_2$ from the Cambridge supplier are a priori reliable. In any case, in order to ensure the accuracy of future biological N_2 fixation determinations, it is recommendable to use the commercial stocks presumably free of contaminants according to Dabundo *et al.* (2014) (Cambridge and Campro Scientific) and to conduct control tests such as those herein described prior to use the $^{15}N_2$ lecture bottles acquired.

SUPPLEMENTARY DATA

Supplementary data can be found at *Journal of Plankton Research* online.

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Supplementary data

Quantifying the overestimation of planktonic N_2 fixation due to contamination of $^{15}N_2$ gas stocks

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S1. Field sampling, hydrographic measurements, inorganic nutrients, chlorophyll *a* and primary production

Between February 2014 and December 2015 we carried out a total of 15 samplings during the NICANOR (Nitrogen fixation and diffusive fluxes in the NW Iberian Peninsula) project. Water samples from 0, 20, 40 and 70 m depth were collected with Niskin bottles on board B/O Lura at station 2 (43.42ºN, 8.44ºW; depth = 80 m) of the RADIALES time-series project (Series temporales de oceanografia en el norte de España, http://www.seriestemporales-ieo.com) (Fig. S1) for the determination of biological N_2 fixation, inorganic nutrients, chlorophyll *a* and primary production. This station is located in the adjacent shelf off Ría de A Coruña (Golfo Ártabro, NW Iberian Peninsula). Profiles of temperature and salinity were obtained with a CTD (Conductivity-Temperature-Depth) probe SBE25plus (SeaBird Electronics) attached to a rosette of Niskin bottles. Mixed layer depth was calculated as the depth where seawater density was 0.125 kg m^{-3} higher than the surface value.

Unfiltered samples for the determination of dissolved inorganic nutrients (NO_3^-) were collected in rinsed polyethylene 15-mL tubes and stored frozen at -20 °C, until further analysis by standard colorimetric methods on a segmented flow AutoAnalyzer 3 Bran Luebbe (Aminot and Kerouel, 2007). Nutrient analyses were performed at the facilities of the University of Oviedo (Spain). Data for nutrient concentrations were not available for December 2014 and April 2015. In these cases, nitrate concentration was computed from a nitrate-density (sigma-t) relationship built by using all samples (n=52) collected during the NICANOR project. The relationship showed a linear behavior (NO_3^- =9.948 x sigma-t −260.850; R^2 =0.930; p<0.05) for sigma-t ranging between 26.1 and 27.1 kg m^{-3} (Moreira-Coello *et al.*, 2017).

Chlorophyll *a* and primary production were determined at 0, 20 and 40 m depth. 150-mL samples were filtered onto Whatman GF/F filters for the total fraction. Filters for chlorophyll *a* quantification were extracted in 90% acetone at 4ºC overnight and measured using the spectrofluorometric method, as described in Neveux and Panouse (1987). Primary production was measured by ^{14}C-uptake. Seawater samples were transferred to triplicate 300–mL polycarbonate bottles (Nalgene) (2 light and 1 dark bottles), which were spiked with 2-10 µCi of $NaH^{14}CO_3$ and incubated during 24 h in refrigerated incubators equipped with a system of recirculating water, and covered with neutral density screens to simulate the corresponding in situ irradiance. Sample filtration, filter processing and primary production calculations were conducted as described in detail in Bode *et al.* (2011).

The results of December 2014 were obtained under hydrographic conditions of winter mixing, which was characterized by a deep mixed layer (51 m), high nitrate concentration (ranging from 3.5 to 6.5 µM), low chlorophyll *a* concentration (ranging between 0.2 and 0.7 mg m^{-3}) and low primary production (ranging from 4.8 to 23.5 mgC m^{-3} d^{-1}) (Fig. S2).

S2. Contamination control test with *Tetraselmis suecica* cultures

This experiment was performed in January 2015. Culture medium was f/2 for all the components (including phosphate, silicate, trace metals and vitamins, but not ammonium) except for nitrate, which was limited to f/32. We used a nitrogen-reduced growth medium to favour the assimilation of the ^{15}N-contaminants presumably contained in some of the $^{15}N_2$ gas stocks. Two different commercial stocks of $^{15}N_2$ gas were used to equilibrate with the culture media: Sigma-Aldrich lot MBBB0968V and Cambridge-Isotope lot I-19168A. According to Dabundo *et al.* (2014), the Sigma lot includes substantial contamination by ^{15}N-labelled nitrate, nitrite and ammonium. We have no data about the composition of the specific Cambridge gas lot used, but other analyzed stocks of the same commercial supplier showed negligible contamination (Dabundo *et al.*, 2014). Two 1-L acid-cleaned clear polycarbonate bottles (Nalgene) were filled with the prepared and autoclaved growth medium using acid-washed silicone tubing and removing all air bubbles. Bottles were closed with caps with silicone septa. Using a gas-tight syringe, we injected 1.5 mL of $^{15}N_2$ gas (98 atom%) from Sigma and Cambridge stocks into each bottle. Then, injected gas and culture medium were equilibrated during 60 h by mixing of the bottles with a rotating mixer (35 rpm). All the material used in this experiment was first autoclaved, except caps with silicone septum, which were UV-sterilized prior to use.

Three 250-mL Erlenmeyer flasks were filled with 200 mL of the growth medium equilibrated with $^{15}N_2$ gas from the Sigma stock, three with medium equilibrated with $^{15}N_2$ from the Cambridge stock, and finally another three with unamended medium (control). The 9 flasks were inoculated with a stock culture of *T. suecica* (28 μg L^{-1} of initial concentration of chlorophyll *a* in each flask) and incubated during four days in a culture chamber at 21°C with an irradiance of 150 μE m^{-2} s^{-1} under a 14:10 (light:dark) photoperiod. Cultures were subjected to aeration through filters of 0.22 μm (Millipore) to stimulate the growth of the microalgae. We took aliquots for the determination of *in vivo* fluorescence and chlorophyll *a* concentration at the beginning of the incubation (day 0), and on days 2, 3 and 4. Filters for chlorophyll *a* quantification were extracted in 90% acetone at 4ºC overnight and measured using the non-acidification technique (Welschmeyer, 1994) with a TD-700 Turner Designs fluorometer calibrated with pure chlorophyll *a*. The nitrate concentration of the cultures was also measured using a colorimetric test (Nitrate Pro Test Kit, Red Sea).

Two days (during the exponential growth phase) and four days (during the nitrate-depleted and stationary growth phase) after inoculation, 30 mL of the cultures were filtered through 25 mm Whatman GF/F filters for the determination of the ^{15}N isotope content in the particulate organic nitrogen (PON) ($\delta^{15}N_{PN}$).

The measurements of *in vivo* fluorescence, chlorophyll *a* and nitrate concentrations allowed us to monitor the evolution of the cultures. On day 2 after inoculation, the microalgae were in exponential growth phase, and on day 4 they had reached the stationary phase and nitrate provided in the medium had been exhausted (Table S1). Because the kinetic isotopic fractionation only occurs while substrates are in excess (Montoya, 2008), we extended the experiment until the nitrate was depleted in order to avoid, to the highest possible extent, fractionation effects during its uptake. We thus tried to ensure that the microalgae assimilated most of the available nitrate. PON from the Cambridge and control cultures was ^{15}N-depleted since the nitrogen source for the microalgae was the synthetic nitrate ($NaNO_3$) added to the growth media. The analysis of this nitrate resulted in a ^{15}N atom% (0.3656±0.0001%) below the atmospheric value of reference (0.3664%, Montoya *et al.*, 1996) and consequently negative $\delta^{15}N$ (–1.9±0.2‰), whereas the $\delta^{15}N$ of deep nitrate from marine waters ranges usually between 3‰ and 6‰ (Sigman *et al.*, 1997, 2000).

S3. Biological N_2 fixation rates

We determined N_2 fixation rates using the $^{15}N_2$-uptake technique (Montoya *et al.*, 1996). Two sets of triplicate 2-L acid-cleaned clear polycarbonate bottles (Nalgene) were filled with water of each depth using acid-washed silicone tubing. After carefully removing all air bubbles, Nalgene bottles

were closed with caps provided with silicone septa, through which 3 mL of $^{15}N_2$ gas (98 atom%) were injected with a gas-tight syringe. Only in December 2014 one set of bottles was injected with gas from the Sigma stock and the other set with the Cambridge gas stock. The pressure across the septum was equilibrated by allowing the excess water to escape through a syringe needle piercing the septum. Incubation bottles were gently shaken by manually inverting them fifty times, and then incubated for 24 h in refrigerated incubators equipped with a system of recirculating water. The incubation of seawater from 70 m (collected in the aphotic zone) was performed under complete darkness, by covering the incubation bottles with black duct tape and placing them inside a black opaque plastic bag. Incubations ended by filtration through 25 mm Whatman GF/F filters to determine $\delta^{15}N_{PN}$ and then total N_2 fixation rates. 2-L seawater samples from each depth were also filtered at time zero for the determination of $\delta^{15}N_{PN}$.

In order to compute the apparent N_2 fixation rate stemmed from the cultures of *T. suecica* equilibrated with contaminated $^{15}N_2$ from Sigma, the difference observed in ^{15}N atom% regarding the control cultures was used (Table S1). This approach consisted in considering *T. suecica* cultures as a natural phytoplankton community to which we measured its N_2 fixation rate. We took into account the salinity (36.5‰) and temperature (21ºC) of the culture medium for the calculations.

S4. $\delta^{15}N_{PN}$ analysis

$\delta^{15}N$ is a parameter that expresses the $^{15}N/^{14}N$ isotopic ratio in a sample in relation to the standard value in the atmospheric N_2. After filtration, filters were dried at 40ºC during 24 h and stored at room temperature until pelletization in tin capsules. Measurements of the ^{15}N atom% of the PON were carried out with an elemental analyser combined with a continuous-flow isotope ratio mass spectrometer (FlashEA112 + Deltaplus, ThermoFinnigan), and using an acetanilide standard as reference. The precision of the analysis, expressed as the standard deviation of the ^{15}N values determined in a series of 10 standards, was 0.15‰. We also measured the nitrogen isotopic composition in three samples of the synthetic salt of nitrate ($\delta^{15}N_{NaNO_3}$) used to prepare the growth media. Isotopic analyses were performed at the stable isotope facility (*SAI*) of the University of A Coruña (Spain).

S5. N_2 fixation rates calculation

Our experiment with *T. suecica* cultures demonstrates that a change of $\delta^{15}N$ of 3‰ between the initial and the final PON of a sample is significant, and given that the detection limit of the used elemental analyser is 0.15-0.20 µg N, the detection limit of our method is approximately 0.001

nmol N L^{-1} d^{-1}. The equations of Weiss (1970) and Montoya *et al.* (1996) were used to calculate the initial N_2 concentration (assuming equilibrium with atmosphere) and N_2 fixation rates, respectively.

S6. Diazotrophs biomass calculation

The calculations of the depth-integrated biomass of diazotrophs that would be explained by the overestimated N_2 fixation rates were computed assuming an average carbon-specific growth rate for different cyanobacterial diazotrophs of 0.3 d^{-1} (Berman-Frank *et al.*, 2007; Mulholland and Bernhardt, 2005; Tuit *et al.*, 2004; Turk-Kubo *et al.*, 2018), balanced growth in terms of carbon and nitrogen, Redfield stoichiometry for the C:N ratio of diazotrophic biomass, and that diazotrophs only use N derived from N_2 fixation.

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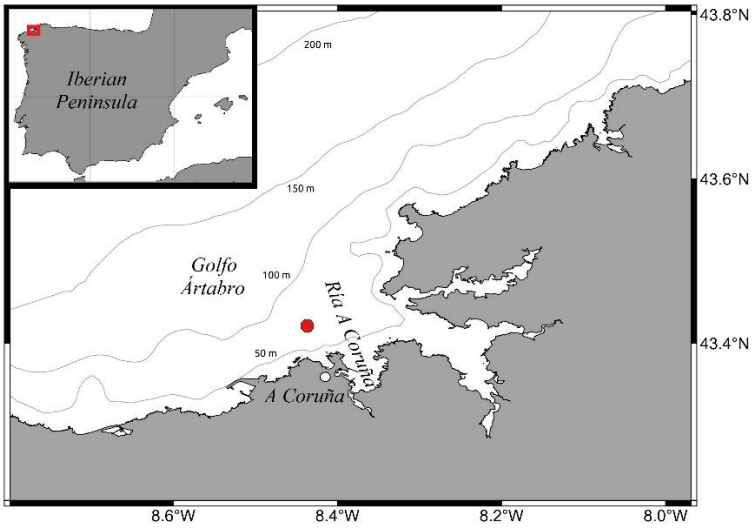

Fig. S1. Sampling station (red dot) located at 80 m depth in the adjacent shelf off Ría de A Coruña (Golfo Ártabro, NW Iberian Peninsula). The 50, 100, 150 and 200 m isobaths are indicated.

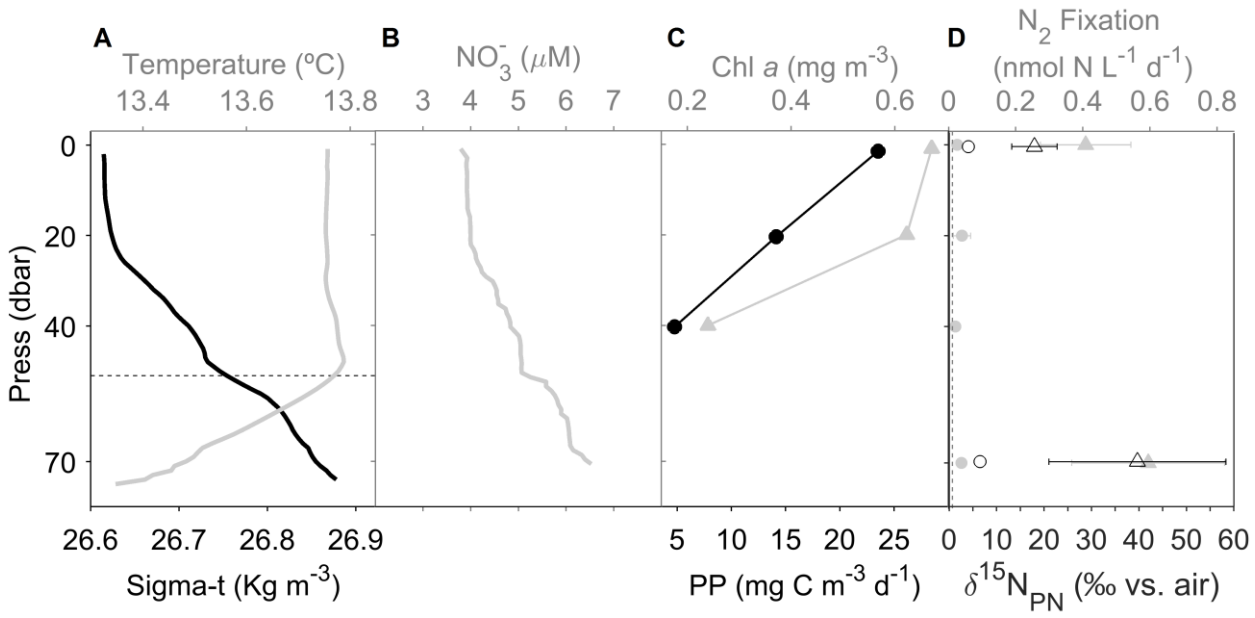

Fig. S2. Vertical distribution of **(A)** temperature and density (sigma-t), **(B)** nitrate concentration computed from a nitrate-density (sigma-t) relationship (see S1 section), **(C)** chlorophyll *a* concentration (Chl *a*) (triangles) and primary production (PP) (circles), and **(D)** biological N_2 fixation rates and $\delta^{15}N$ of the particulate organic nitrogen ($^{15}N/^{14}N$ isotopic ratio in the sample in relation to the standard value in the atmospheric N_2) by using contaminated $^{15}N_2$ gas from Sigma-Aldrich stock (filled triangles and empty triangles, respectively) and the presumably contaminant-free Cambridge-Isotope stock (filled circles and empty circles, respectively), sampled in December 2014. Error bars represent standard deviation. The dashed line in the sigma-t and N_2 fixation panels indicate the mixed layer depth (estimated from an increase in water column density of 0.125 kg m^{-3} relative to surface values) and the limit of detection of the N_2 fixation rates, respectively.

Table S1. Mean (±SD) of in vivo fluorescence, chlorophyll *a*, nitrate concentration, ^{15}N content of the particulate organic nitrogen ($\delta^{15}N_{PN}$, ‰) and ^{15}N atom% in the different treatments of *T. suecica* cultures. Asterisk indicates initial nitrate concentration in the f/32 medium culture. ND: not detectable.

t (day)	Treatment	*In vivo* fluo (fsu)	Chl *a* (µg L^{-1})	NO_3^- (µM)	$\delta^{15}N_{PN}$ (‰ vs. air)	^{15}N atom%
0	**Control Cambridge Sigma**	32	28	55*	-	-
2	**Control**	61±1	127±27	-	-0.90±0.01	0.3660±0.0001
	Cambridge	61±14	120±24	-	-1.0±0.2	0.3659±0.0001
	Sigma	59±2	98±17	-	1.7±0.3	0.3669±0.0001
3	**Control**	113±3	157±16	-	-	-
	Cambridge	118±3	149±3	-	-	-
	Sigma	110±1	166±9	-	-	-
4	**Control**	155±13	120±5	ND	-1.4±0.3	0.3658±0.0001
	Cambridge	178±6	101±11	ND	-1.3±0.2	0.3658±0.0001
	Sigma	169±10	105±14	ND	1.5±0.2	0.3669±0.0001