Low contribution of \( \text{N}_2 \) fixation to new production and excess nitrogen in the subtropical northeast Atlantic margin

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Abstract

We used $^{15}$N-labeled substrates to measure dinitrogen ($N_2$) fixation, nitrate ($NO_3^-$) and ammonium ($NH_4^+$) uptake, regeneration and associated dissolved organic nitrogen (DON) release in a coastal upwelling system (Cape Ghir, ~31°N) and an open ocean grid (bounded between 25°– 42°N and 20°W) in the Canary Current region during the summer of 2009. New production ($P_{new} = NO_3^-\text{uptake} + N_2\text{fixation} + \text{DON released from NO}_3^-\text{uptake} - NO_3^-\text{regeneration}$) was higher in the upwelling than in the open ocean zone (0.126 and 0.014 µmol N L$^{-1}$ h$^{-1}$, respectively), while regenerated production ($P_{reg} = NH_4^+\text{uptake} + \text{DON released from NH}_4^+\text{uptake} + NH_4^+\text{regeneration}$) was similar in both zones (0.157 and 0.133 µmol N L$^{-1}$ h$^{-1}$, respectively). The resulting f-ratio ($P_{new}/P_{new}+P_{reg}$) for the open ocean and upwelling zones was 0.08 and 0.48, respectively. The availability of nitrogen in excess of that expected from Redfield stoichiometry is generally attributed to $N_2$ fixation. A previous study indicated that our open ocean grid zone had an excess nitrogen production rate of $40 \pm 22 \times 10^{10}$ mol N y$^{-1}$. We revisited this budget including new dissolved organic matter and NO$_3^-$ fluxes through the Strait of Gibraltar and estimated a revised nitrogen excess rate of $22 \pm 19 \times 10^{10}$ mol N y$^{-1}$. The average volumetric rate of $N_2$ fixation for this zone was only $1.3 \times 10^{-3}$ nmol N L$^{-1}$ d$^{-1}$, indicating that its influence in $P_{new}$ and nitrogen excess production in this part of the Atlantic is negligible.

Key words: New production, regenerated production, f-ratio, diazotrophy, upwelling, dissolved organic nitrogen release
1. Introduction

One of the greatest current challenges for oceanographers is to balance the oceanic nitrogen budget. At present, it is estimated that nitrogen losses exceed global nitrogen fixation in the upper water column by ~14 × 10^{12} mol N y^{-1} (Codispoti, 2007). Massive combined nitrogen losses through denitrification and anammox in sediments and oxygen minimum zones of the eastern tropical Pacific Ocean push the global budget towards net loss (Deutsch et al., 2001), although the opposite may be found in other basins. In the North Atlantic Ocean, where pelagic denitrification is thought to be negligible (Hansell et al., 2007), excess fixed nitrogen —calculated under the assumption of a Redfieldian nitrogen to phosphorus (N:P) molar ratio of 16— has been repeatedly observed with varying rates: 0.15 – 0.46 × 10^{12} mol N y^{-1}, 0.8 × 10^{12} mol N y^{-1}, 2 × 10^{12} mol N y^{-1}, and 2 – 9 × 10^{12} mol N y^{-1} by Hansell et al. (2004, 2007), Gruber and Sarmiento (1997), and Capone et al. (2005), respectively.

Dinitrogen (N_2) fixation is a biologically mediated process that reduces N_2 to ammonia via the nitrogenase enzyme complex, carried by several species of cyanobacteria, bacteria and some Archaea (Riemann et al., 2010). N_2 fixation uses phosphate (HPO_4^{2-}) but not combined inorganic nitrogen, leaving a signal of excess nitrogen mineralization compared with the expected Redfield’s N:P ratio of 16 in the 70–700 m layer of the North Atlantic Ocean (Mahaffey et al., 2005). This excess nitrogen accumulation is commonly traced by the N* parameter, following the general formulation N* = [NO_3^-] – 16×[HPO_4^{2-}], where [NO_3^-] and [HPO_4^{2-}] are the concentrations of nitrate and HPO_4^{2-}, respectively. This equation has been
amended by adding constants that bring the global N:P ratio to 16 and the intercept to zero (Gruber and Sarmiento, 1997; Hansell et al., 2004, 2007). Note that the N* parameter theoretically measures net \( \text{N}_2 \) fixation, i.e. the resulting excess or deficit of \( \text{NO}_3^- \) resulting from the difference between denitrification and \( \text{N}_2 \) fixation in the mesopelagic ocean.

Excess nitrogen production can also be estimated using biological approaches, which include the direct measurement of biological \( \text{N}_2 \) fixation rates using the acetylene reduction assay (ARA; Stal, 1988) or the stable isotope \( ^{15}\text{N} \) (Montoya et al., 1996; Mohr et al., 2010). Geochemically estimated rates usually exceed biologically estimated rates by one order of magnitude (Mahaffey et al., 2005). This discrepancy among methods impedes global oceanic nitrogen balancing based on the available data. There are a number of reasons why such differences exist between methods. While geochemical methods attempt to measure \( \text{N}_2 \) fixation rates in large oceanic areas (e.g. basin scale) and large periods of time (e.g. years), biological methods are based on bioassays performed on small volumes (usually from a few mL to 5 L), and short periods of time (up to 24 h). There is also a lag of time and space between the biological process and its geochemical signature. The organic matter produced by \( \text{N}_2 \) fixation with a high N:P in the epipelagic layer sinks to the mesopelagic layer where it is remineralized, leaving the characteristic excess nitrogen signature catch by the N* parameter. Nitrogen anomalies are maximum in the thermocline and travel away from the area where \( \text{N}_2 \) fixation occurs along isopycnal surfaces, making gradients in nitrogen excess the only way to localize the production areas (Mahaffey et al., 2005). Moreover, Redfield stoichiometry is generally assumed in these geochemical calculations (C:N:P = 106:16:1),
while in practice particulate organic matter (POM), and especially dissolved organic matter (DOM), can often have C:N:P ratios well above Redfield (e.g. Vidal et al., 1999; Doval et al., 2001; Hopkinson and Vallino, 2005). Although new methodological improvements may bring geochemical and biological rates closer in the future (Mohr et al., 2010; Großkopf et al., 2012), the nitrogen anomaly found in the North Atlantic Ocean remains unexplained to date.

In the eastern subtropical North Atlantic, Álvarez and Álvarez-Salgado (2007) calculated an excess of $40 \pm 22 \times 10^{10}$ mol N y$^{-1}$ for the region bounded by 25º–42ºN and 20ºW. These authors tentatively attributed this excess to N$_2$ fixation, but the lack of in situ measurements in the area could not confirm it by that time. The aim of this paper is to verify if the quantified excess nitrogen could be supported by N$_2$ fixation and to assess the role of N$_2$ fixation in nitrogen cycling in this area during the summer, when N$_2$ fixation rates are presumably maxima due to the higher temperatures. With this purpose, we measured NO$_3^-$ and ammonium (NH$_4^+$) uptake and regeneration, and the associated dissolved organic nitrogen (DON) release in the upper water column of this study area, and compared it to the N$_2$ fixation measurements reported in Benavides et al. (2011) for the same cruise.

2. Material and methods

2.1. Sampling and hydrographic measurements

Sampling was performed from 25 July to 5 September 2009 during the ‘Shelf-Ocean Exchanges in the Canaries-Iberian Large Marine Ecosystem’ (CAIBEX) cruises, onboard
R/V *Sarmiento de Gamboa*. A total of 73 stations were sampled in an open-ocean grid which
covered the area between the northwest (NW) Iberian Peninsula and the Canary Islands,
drawing a box enclosed by the 20°N meridian (the ‘CAIBOX’ grid; Figure 1a). In 17 of the
stations (white dots in Figure 1a), samples for inorganic nutrients, dissolved organic carbon
(DOC), and total dissolved nitrogen (TDN) analyses were taken at 15 levels, from surface to
bottom. In these 17 stations, samples for the determination of N₂ fixation rates were collected
at the surface (5 m). At even stations of the open-ocean grid (Figure 1a, stations X2, X4, X6,
X8, X10, X12 and X16) samples for the determination of nitrogen uptake and regeneration
and DON release rates were taken a two selected depths: surface (5 m) and the deep
chlorophyll maximum (DCM). In addition, inorganic nutrients, DOC, TDN and nitrogen
metabolic rates were also measured in another 8 stations in the upwelling region off Cape
Ghir, in the NW coast of Africa (Figure 1b). Hereafter, these two areas will be operationally
referred to as ‘non upwelling’ and ‘upwelling’-affected, respectively.

At each station, temperature, salinity and photosynthetically active radiance (PAR) data
were recorded by means of a SBE 911 plus CTD, equipped with a Sea-Tech fluorometer and
Li-Cor PAR sensor. Temperature and salinity were also obtained by means of a Seasoar
towed undulating system (Chelsea Technologies Group), fitted with a SBE 911 CTD. The
Seasoar was towed over the study area performing six transects parallel to the coast, and
undulating between 10 and 400 m depth. The near-surface (~5 m) temperature and salinity
data were obtained from the flow-through system of the ship by means of a SBE 21 Seacat
Thermosalinograph.
2.2. Dissolved inorganic and organic nitrogen and carbon analyses

Seawater was collected using 12 L Niskin bottles mounted on a General Oceanics rosette sampler. In the non upwelling-affected stations, samples for inorganic nutrient analyses were drawn into 50 mL polyethylene containers (VWR) and immediately analyzed on-board using a Prescop Alpkem autoanalyzer with detection limits of 0.05 µM for NO$_3^-$ and Si(OH)$_4$, and 0.02 µM for NO$_2^-$ and HPO$_4^{2-}$. In the upwelling-affected stations, samples were recovered in 15 mL polyethylene tubes (VWR) and stored frozen at -20ºC. These samples were analyzed in the land-based laboratory using an AA3 Bran+Luebbe autoanalyzer with detection limits of 0.01 and 0.03 µM for NO$_3^-$ and NO$_2^-$, respectively. Samples for the determination of the NH$_4^+$ concentration in the surface (5 m) and DCM depth were directly sampled from the rosette into 100 mL borosilicate bottles (Duran Schott) and measured on board with a Perkin-Elmer LS55 spectrofluorometer following the method by Holmes et al. (1999). The detection limit for this method is 5 nM.

Samples for DOC and TDN analyses in the upper 150 m were filtered through Whatman GF/F filters in an acid-cleaned all-glass filtration system under positive pressure of high purity N$_2$. The filtrate was collected in 10 mL precombusted (450ºC, 6 h.) glass ampoules. Samples from below 150 m were directly collected in the glass ampoules. After acidification to pH< 2 with phosphoric acid, the ampoules were flame-sealed and stored in the dark at 4ºC until analysis. They were measured with a nitrogen-specific Antek 7020 nitric oxide chemiluminescence detector coupled in series with the carbon-specific infrared gas
analyzer of a Shimadzu TOC–5000 organic carbon analyzer (Álvarez–Salgado and Miller 1998). The measurement error was ± 0.7 µM for carbon and ± 0.3 µM for nitrogen. Their respective accuracies were tested with the reference materials provided by Prof. D.A. Hansell (Univ. of Miami), which were run once per day just after calibration with a mixed standard of potassium hydrogen phthalate and glycine. DON was obtained by subtracting NO$_3^-$, NO$_2^-$ and NH$_4^+$ (when available) from TDN.

2.3. Calculation of the $N^*$ parameter and chlorophyll a concentrations

Chlorophyll a concentrations were estimated fluorometrically by means of a Turner Designs bench fluorometer, previously calibrated with pure chlorophyll a (Sigma), according to Holm-Hansen et al. (1965). Seawater samples (500 mL) were filtered through Whatman GF/F filters. Pigments were extracted in cold acetone (90% v/v) for 24 hours. The regression expression between fluorescence and chlorophyll a values was: chlorophyll a = 0.3531 × F + 0.0983 for the non upwelling-affected area (CAIBOX) and chlorophyll a = 3.3797 × F + 0.0279 for the upwelling-affected area (Cape Ghir), with chlorophyll a expressed in mg m$^{-3}$. The correlation coefficient was $r^2 = 0.88$ and 0.81, for CAIBOX and Cape Ghir, respectively.

Following the equation proposed by Gruber and Sarmiento (1997) we calculated $N^*$ as:

$$N^* = ([\text{NO}_3^-] - 16[\text{PO}_4^{3-}] \times [\text{HPO}_4^{2-}] + 2.9) \times 0.87,$$

where [NO$_3^-$] is the concentration of NO$_3^-$ in µmol kg$^{-1}$, [HPO$_4^{2-}$] is the concentration of phosphate in µmol kg$^{-1}$, and 2.9 and 0.87 are a constant and a multiplier, respectively, used to force the global average $N^*$ to zero. A section of this parameter over the non upwelling-affected (CAIBOX) region is shown in Figure 4a.
2.4. Nitrogen uptake, nitrogen regeneration and DON release

$\text{NO}_3^-$ and $\text{NH}_4^+$ uptake, regeneration and associated release as DON were measured at the surface (5 m) and DCM depth of stations X2, X4, X6, X8, X10, X12, X14 and X16 in the non upwelling-affected area (Figure 1a), and at all stations of the upwelling-affected area (Figure 1b). For $\text{\textsuperscript{15}N}$ experiments, water was directly transferred from the Niskin bottles to 2 L transparent polycarbonate bottles (Nalgene) in duplicate. $\text{\textsuperscript{15}N}$-labeled substrate was added to the bottles as 1 mL of $\text{\textsuperscript{15}KNO}_3$ (200 $\mu$M; 99 at.%), or 1 mL of $\text{\textsuperscript{15}NH}_4\text{Cl}$ (20 $\mu$M; 99 at.%) (Sigma-Aldrich). For the non upwelling-affected stations, isotope enrichments were on average 22 and 27% for $\text{NO}_3^-$ and $\text{NH}_4^+$, respectively. In the case of the upwelling-affected stations, average enrichments were 5 and 56% for $\text{NO}_3^-$ and $\text{NH}_4^+$, respectively.

Replicate bottles were immediately filtered after inoculation of the labeled substrates to obtain the initial $\text{\textsuperscript{15}N}$ enrichment of samples. The other set of replicate bottles were equally inoculated and placed in on-deck incident light-calibrated containers cooled with surface seawater for 3–4 h. A hand light-meter (Biospherical Instruments) and neutral density filters (Lee filters) were used to mimic the light levels measured on PAR profiles at each station. After the incubation period, the samples were gently filtered through precombusted GF/F filters (vacuum pressure <100 mm Hg), wrapped in precombusted aluminium foil and stored at –20°C. The uptake of $\text{NO}_3^-$ was calculated according to the equations in Dugdale and Wilkerson (1986). $\text{NH}_4^+$ uptake rates were corrected for isotopic dilution as indicated by Glibert et al. (1982) (the equations are displayed in Table 1). Wherever under detection limit
NH$_4^+$ concentrations were observed, values were substituted by the detection limit of the method (i.e. 5 nM), resulting in 50% enrichments, which can be considered as the threshold between real and potential uptake rates (Fernández et al., 2009).

The filtrates were kept to analyze final NO$_3^-$, NO$_2^-$, NH$_4^+$ and DON concentrations (see above), and the $^{15}$N-enrichment of the final NO$_3^-$, NH$_4^+$ and DON pools using the protocol proposed by Slawyk and Raimbault (1995). For the latter, 80 mL of filtrate were stored in 100 mL borosilicate flasks (Duran Schott) and poisoned with 1% HgCl$_2$ (Sigma-Aldrich) to interrupt any microbial activity. The extraction protocol consists of three steps: (1) NO$_3^-$ and NO$_2^-$ are reduced and stripped off together with initial NH$_4^+$ as ammonium sulfate, (2) oxidation of the remaining dissolved nitrogen (i.e. the DON pool) to NO$_3^-$ by persulfate oxidation (Valderrama, 1981), and (3) repetition of step (1) to strip off DON as ammonium sulfate. From the samples incubated with $^{15}$NO$_3^-$, rates of NO$_3^-$ uptake and DON release from NO$_3^-$ are obtained. From the samples incubated with $^{15}$NH$_4^+$, rates of NH$_4^+$ uptake, NO$_3^-$ regeneration, NH$_4^+$ regeneration and DON release from NH$_4^+$ are obtained. Nitrogen regeneration rates (NO$_3^-$ and NH$_4^+$) were calculated by applying the equations in Fernández and Raimbault (2007). DON release rates from NO$_3^-$ and NH$_4^+$ uptaken by microorganisms were calculated with the equations in Slawyk et al. (1998) (see a summary of all equations used in Table 1).

N$_2$ fixation was assayed only in the surface (~5 m) depth as outlined in Benavides et al. (2011), at stations X1 to X17 in the non upwelling-affected area, and at all stations in the upwelling-affected area. Briefly, seawater was transferred to 1 L transparent polycarbonate
bottles (Nalgene). The bottles were filled to overflow using silicone tubing and preventing the introduction of air bubbles. Then they were sealed with septum screw caps before 2 mL of $^{15}\text{N}_2$-gas (99 at. %; Tracer Tec) were injected through the septum using a gas-tight syringe (Hamilton). The pressure across the septum was equilibrated by allowing the excess air to escape through a sterile syringe tip piercing the septum at the same time as the $^{15}\text{N}_2$-gas was being injected. Finally, the bottles were placed in the on-deck incubator for 24 h. After the incubation, samples were filtered through precombusted GF/Fs (vacuum pressure <100 mm Hg), wrapped in precombusted aluminium foil and stored at $-20^\circ\text{C}$ until analysis. $\text{N}_2$ fixation rates were calculated after Montoya et al. (1996) (see Table 1).

The concentration of particulate organic nitrogen (PON) and the isotopic ratio ($^{15}\text{N}/^{14}\text{N}$) of samples was obtained by means of a Thermo Flash 1112 elemental analyzer interfaced by a Conflo III with a Thermo Delta V Advantage isotope ratio mass spectrometer (IRMS).

3. Results

3.1. Hydrography and chlorophyll a

Sections of temperature and salinity in the upper 200 m of the open ocean transect (non-upwelling-affected area, Figure 1a) are shown in Figure 2. Temperature increased southwards along the transect (Figure 2a). A sharp thermocline was observed in the northern part of the transect (stns X2 to X6, Figure 1a), where temperatures of 19–20$^\circ\text{C}$ were measured in the upper 40 m, then decreasing to 15–17$^\circ\text{C}$ at 50–60 m depth. The Azores Front was located slightly north of stn X8 and can be clearly identified by the tilting in the isotherms. South of
the Azores Front, the entire water column was warmer and temperatures >19°C were observed down to ~80 m depth. This pattern was only disrupted south of stn X16, when reaching the coastal transition zone (CTZ) between the NW African upwelling ecosystem and the Canary Islands (Figure 1b). Salinity mimics the structure of temperature in the upper 200 m of the open ocean transect (Figure 2b). In the northern part of this transect, salinity decreased sharply from 38 to ~36.5 in the upper ~60 m depth. The Azores Front was also easily depicted by the tilting isohalines observed near to stn X8. South of the Azores Front, the isohalines deepened (38 at ~100 m depth) and a stratified area was observed from stns X8 to X16. Stratification disruption associated with the CTZ was also observed in the isohalines (Figure 2b). Chlorophyll a concentrations were higher (0.45 to 1 µg l⁻¹) between 50 and 100 m of depth from the beginning of the transect to the Azores Front (located at ~1500 km section distance; Figure 2c). Lower values (<0.2 µg l⁻¹) were observed south of the Azores Front, coinciding with the highest temperatures and salinities observed during this transect (Figs. 2a-b).

Figure 3 shows the temperature, salinity and chlorophyll a maps in the upwelling-affected area near the surface (5 m) and at a depth representative of the DCM at most stations (50 m, see Table 2). The maps near the surface underline the presence of an upwelling filament with an extension of more than 150 km and characterized with low temperatures (16-18°C) and low salinity (~36.2). The maps near the surface underline the presence of an upwelling filament with an extension of more than 150 km and characterized with low temperatures (16-18°C) and low salinity (~36.2), which extended over the shelf from the
northern part of the NW African coast and the sampled stations. The lowest temperature at 5 m (<17°C) was observed at ~31°N, in the core of the coastal upwelling (Figure 3a). An area of warmer (20-21°C) and saltier water (36.5–36.6) was observed leeward of the upwelling-affected stations, over the bay of Agadir (Morocco), probably because of lower wind intensity in this region. This area of warmer waters was also visible in the satellite images (see Figure 1 in Benavides et al., 2011). At 5 m depth, low values of chlorophyll $a$ (<0.5 µg L$^{-1}$) extended towards the offshore zone. Higher concentrations were detected north of the filament area (Figure 3e) and southwest of it too. However, the lack of data points in the southwestern part of the graph suggests that these high concentrations should be taken carefully.

At 50 m depth, the filament decreased in width. The coldest temperatures (15°C) were observed near the coast leeward of the upwelling-affected stations. Stations G12, G17 and G22 were clearly situated over the path of the filament, were temperatures at 50 m depth ranged from 17 to 17.5°C. Station GT2 was situated at the edge of the filament, and stations G40, G44 and G48 at its tip-end, where higher temperatures (17.5 to 18°C) were measured. High values (>1 µg L$^{-1}$) of chlorophyll $a$ at 50 m were detected mainly close to the coast (Figure 3f). Similarly to the surface, relatively high values were observed north and south of the filament area (Figure 3f).

3.2. Inorganic nitrogen and $N^*$ distributions

In situ surface NO$_3^-$ concentrations were generally <1 µmol kg$^{-1}$ at all the stations.
sampled in both study areas with few exceptions (Table 2). These surface concentrations were fairly stable along the non upwelling-affected stations (at ~0.1 μmol kg\(^{-1}\)), being somewhat more variable at the DCM. Peaks of NO\(_3^-\) were measured in the upwelling-affected area associated with the coastal upwelling (stn GM2), and stations over the upwelling filament (G17 and G44). In the non upwelling-affected area, DCM NO\(_3^-\) concentrations equaled those of the surface at most stations, while in the upwelling-affected area increases of up to 1 μmol kg\(^{-1}\) were observed with respect to surface values. NH\(_4^+\) concentrations were under the detection limit at several stations (indicated as n/d in Table 2). Where detectable, NH\(_4^+\) concentrations were < 50 nM in most cases, being somewhat higher north of the Azores Front (stns X2 to X8). NH\(_4^+\) concentrations at the DCM were generally higher than at the surface.

The section of N\(^*\) along the non upwelling-affected area (CAIBOX) revealed relatively high N\(^*\) values (~3–4 μmol kg\(^{-1}\)) spread all over the upper 1500 m of the water column between the Galician coast and stn X4 (Figure 1a, Figure 4). South of the Azores Front (located at ~1200 km section distance), high N\(^*\) values were observed in the layers comprised between the 26.5 and ~27.5 kg m\(^{-3}\) isopycnals (250 to ~800 m), until ~2000 km section distance. The signal then deepened southwards reaching a depth of 1500 m depth in the last stations of the transect (Figure 4).

3.3. Nitrogen uptake, regeneration and release

Surface and DCM NO\(_3^-\) uptake, NH\(_4^+\) uptake, NO\(_3^-\) regeneration, NH\(_4^+\) regeneration,
DON release from uptaken NO$_3^-$ and NH$_4^+$, N$_2$ fixation, and the associated new and regenerated production rates (Pnew and Preg, respectively) were averaged for the non upwelling-affected and upwelling-affected areas (Table 3). Note that N$_2$ fixation rates are only available for the surface. NO$_3^-$ uptake was two orders of magnitude lower than NH$_4^+$ uptake in the non upwelling-affected area, while these were similar in the upwelling-affected area (Table 3). The regeneration of NO$_3^-$ was low in the non upwelling-affected area and not detectable in the upwelling-affected area. NH$_4^+$ regeneration was one order of magnitude lower than NH$_4^+$ uptake in the non upwelling-affected area, and two orders of magnitude lower in the upwelling-affected area. DON release from NO$_3^-$ was similar in both areas, while DON release from NH$_4^+$ was one order of magnitude greater in the upwelling-affected area than in the non upwelling-affected area. N$_2$ fixation was very low in both areas, being somewhat higher in the upwelling-affected area.

Pnew was corrected by adding N$_2$ fixation -as another source of new nitrogen-, and by subtracting NO$_3^-$ regeneration -a source of regenerated nitrogen- (Raimbault and Garcia, 2008). Preg was corrected by adding NH$_4^+$ regeneration to NH$_4^+$ uptake. DON released from NO$_3^-$ or NH$_4^+$ uptake was added to Pnew and Preg, respectively (considering the ‘loss’ of inorganic nitrogen to the DON pool; Raimbault and Garcia, 2008). Thus, the f-ratio was calculated as follows: 

\[ f\text{-ratio} = \frac{(\text{NO}_3^- \text{uptake} + \text{DON release from NO}_3^- + \text{N}_2 \text{fixation} - \text{NO}_3^- \text{regeneration})}{(\text{NO}_3^- \text{uptake} + \text{N}_2 \text{fixation} - \text{NO}_3^- \text{regeneration} + \text{NH}_4^+ \text{uptake} + \text{DON release from NH}_4^+ + \text{NH}_4^+ \text{regeneration})} \]

The NO$_3^-$ regeneration rates measured were low or undetectable in the two sampled areas, so its effect in reducing Pnew was minimal. Similarly,
N₂ fixation rates were several orders of magnitude lower than NO₃⁻ uptake, therefore N₂ fixation usually contributed <1% to Pnew (Table 3). Pnew was much greater than Preg in the upwelling affected area, while they were similar in the non upwelling-affected area. Accordingly, the f-ratio was very low in the non upwelling-affected oligotrophic waters (0.08), and higher (0.48) in the upwelling area (Table 3).

If we consider gross NO₃⁻ and gross NH₄⁺ uptake as NO₃⁻ uptake plus DON release from NO₃⁻, and NH₄⁺ uptake released from NH₄⁺ (Bronk et al., 1994), we find that DON release from NO₃⁻ is more important in the non upwelling-affected area, while DON release from NH₄⁺ is more important in the upwelling-affected area (Table 4). Nevertheless, the variability was very high (note high standard deviation values in Table 4).

4. Discussion

4.1. Uptake and regeneration of NO₃⁻ and NH₄⁺ and associated DON release in open ocean and upwelling areas

It is generally assumed that primary production in upwelling systems is mainly supported by NO₃⁻ ('new nitrogen'), which is brought to the surface through the advection of nutrient-rich deep waters to shallower coastal areas. Instead, productivity in open ocean oligotrophic ecosystems is thought to rely on NH₄⁺ ('regenerated nitrogen') due to the year-round persistence of a strong thermocline that prevents nutrient-rich deep waters to reach the photic layer (Dugdale and Goering 1967). The f-ratio quantifies the contribution of Pnew to total production (Pnew + Preg) (Eppley and Peterson, 1979).
Upwelling systems are expected to have high f-ratios, while oligotrophic systems usually present low f-ratios. Nevertheless, the recent inclusion of N\textsubscript{2} fixation, NH\textsubscript{4}\textsuperscript{+} and NO\textsubscript{3}\textsuperscript{-} regeneration and DON release rates in the computation of f-ratios has shown that P\textsubscript{new} is generally overestimated when nitrification is not taken into account, while P\textsubscript{reg} is generally underestimated when NH\textsubscript{4}\textsuperscript{+} regeneration is not taken into account (e.g. Fernández and Raimbault, 2007), while gross NO\textsubscript{3}\textsuperscript{-} and NH\textsubscript{4}\textsuperscript{+} uptake rates are underestimated if the associated DON released is not accounted for (e.g. Bronk et al., 1994). In this study, all the above-mentioned fluxes have been included in the calculation of P\textsubscript{new}, P\textsubscript{reg} and f-ratios, enhancing the robustness of our results.

The NO\textsubscript{3}\textsuperscript{-} uptake rates in the upwelling-affected zone were in the range of those observed in the upwelling systems off California (0.10 – 0.55 µmol N L\textsuperscript{-1} h\textsuperscript{-1}; Dugdale et al., 2006), and off Peru (0.11 – 0.14 µmol N L\textsuperscript{-1} h\textsuperscript{-1}; Blasco et al., 1984), although more recently, rates one to two orders of magnitude lower (0.003 – 0.02 µmol N L\textsuperscript{-1} h\textsuperscript{-1}) have been reported for the Peruvian and Chilean upwelling systems (Raimbault and Garcia, 2008; Fernández et al., 2009). While these authors observed considerably high NO\textsubscript{3}\textsuperscript{-} regeneration (nitrification) rates in the surface waters of the South Pacific Ocean upwelling systems (0.125 – 1.0 × 10\textsuperscript{-3} µmol N L\textsuperscript{-1} h\textsuperscript{-1}), this flux was not detectable during our study in the coastal upwelling off Cape Ghir. In the non-upwelling affected zone (CAIBOX), NO\textsubscript{3}\textsuperscript{-} uptake and regeneration rates were similar to that observed in a study in the same longitudinal range, but slightly to the north (between ~38 and 45°N) by Fernández and Raimbault (2007), but one to two orders of magnitude higher than previously observed off the coast of Portugal in an area included in
Generally, we observed that our NH$_4^+$ uptake rates were considerably higher than reported in other studies. For example, Fernández et al. (2009) measured NH$_4^+$ uptake rates ranging from $0.59 \times 10^{-3}$ to 0.006 µmol N L$^{-1}$ h$^{-1}$ off the Peruvian upwelling system, and Raimbault and Garcia (2008) measured a maximum of 0.02 µmol N L$^{-1}$ h$^{-1}$ off the Chilean upwelling system. In an open ocean area of the North Atlantic, Fernández and Raimbault (2007) measured NH$_4^+$ uptake rates up to ~0.01 µmol N L$^{-1}$ h$^{-1}$. All these rates are two to three orders of magnitude lower than our NH$_4^+$ uptake rates measured in the upwelling-affected and non upwelling-affected areas (Table 3). However, it should be noted that the standard deviation values of our NH$_4^+$ uptake rates were considerably high (Table 3), and that non-detectable NH$_4^+$ concentrations where substituted by 5 nM (the detection limit of the method used; Holmes et al., 1999), and therefore those rates can be considered as ‘potential’ NH$_4^+$ uptake rates (Fernández et al., 2009), potentially overestimating the Preg rates obtained here.

The NH$_4^+$ regeneration rates measured in this study fall within the range observed in other upwelling and open ocean areas. For example, Fernández et al. (2009) measured NH$_4^+$ regeneration rates ranging from 0.006 to 0.02 µmol N L$^{-1}$ h$^{-1}$ off the coast of Peru, and our rates are also in the range of those observed in an upwelling-affected area off the Galician coast (maximum 0.03 µmol N L$^{-1}$ h$^{-1}$; Varela et al., 2003). Similarly, the rates of NH$_4^+$ regeneration observed in the non upwelling-affected area are in the range of those observed in the North Atlantic by Fernández and Raimbault (2007) (up to ~0.02 µmol N L$^{-1}$ h$^{-1}$).
In our study, Preg rates were similar in the upwelling-affected and non upwelling-affected zones, corroborating the importance of nitrogen regeneration in sustaining primary production (e.g. Fernández and Raimbault, 2007; Clark et al., 2011; Fernández and Farías, 2012). As previously mentioned, open ocean oligotrophic zones are expected to have higher Preg rates than coastal upwelling zones, a pattern typically related to nutrient availability and the related planktonic community structure: picoplankton dominates in oligotrophic areas (e.g. Zubkov et al., 2000), while autotrophic microplankton such as diatoms usually dominate in active upwelling systems (e.g. Hutchings et al., 1995). In contrast, pico- and nanoplanktonic cells predominate the NW Iberian and NW African upwelling systems, whereas diatoms only thrive during upwelling pulses (Espinoza-González et al., 2012; Anabalón et al., unpubl.). In a study performed in the same time of the year, Baltar et al. (2009) found high abundances of heterotrophic prokaryotes (up to $1.5 \times 10^7$ cells mL$^{-1}$) in an area where the upwelling filament stretching off Cape Jubi (south of Cape Ghir, off the NW African coast) interacts with the oligotrophic open ocean waters. Similarly, Raimbault and Garcia (2008) observed high NH$_4^+$ regeneration rates in the upwelling off Chile, associated with the high abundance of heterotrophic plankton. Another piece of evidence is the increasing surface DOC concentrations observed as we move offshore (Table 2), which indicate an enhancement of microbial loop processes, as previously seen in the coastal upwelling zone off Cape Ghir García-Muñoz et al. (2005).

The DON release rates observed in our study were in the order of those previously measured by Varela et al. (2003) off the Spanish Atlantic coast, but usually an order of
magnitude higher than those observed in other oceanic zones (see Table 8.9. in Bronk and Steinberg, 2008). If we consider gross nitrogen uptake as net nitrogen uptake (nitrogen incorporated into biomass) plus nitrogen ‘lost’ as DON, we can calculate the percentage contribution of DON release to gross nitrogen uptake (Bronk et al., 1994). The percentage contributions of DON release to gross NO$_3^-$ and gross NH$_4^+$ uptake observed (Table 4) were in the range of those previously measured in the Atlantic (Varela et al., 2005). DON release derived from NO$_3^-$ predominated in non upwelling-affected zone, while DON released from NH$_4^+$ predominated in the upwelling-affected zone (Table 3). This discrepancy between open ocean and coastal zones has been observed in a range of studies (reviewed by Bronk and Steinberg, 2008).

4.2. Distribution of N* and comparison with in situ $N_2$ fixation

The distribution of N* suggests a possible link to in situ $N_2$ fixation in the southern part of the transect, where higher N* values were observed (Figure 4a), coinciding with the highest $N_2$ fixation rates (see Benavides et al., 2011). However, the high and water column-spread N* values in the northern part of the transect do not correspond with high $N_2$ fixation rates (Figure 4a; Benavides et al., 2011). Note that the link between the process ($N_2$ fixation) and the signal (high N*) is not direct as there is usually a lag time of several weeks to months between $N_2$ fixation in the epipelagic layer and the N* positive signal indicative of $N_2$ fixation in the mesopelagic layer (Mahaffey et al., 2005; Singh et al., 2013). Moreover, the N* signal travels away from the formation zone along isopycnal surfaces (Mahaffey et al.,
2005), which gives room for the possibility that the N* signals observed here had been transported from elsewhere. For example, Palter et al. (2011) suggest that the positive N* signature generated south of the Gulf Stream is transported eastwards through the North Atlantic Current. Alternatively, the positive N* signal may be related to the preferential consumption of phosphorus over nitrogen (see discussion subsection 4.3.), or to non-Redfieldian ratios in the dissolved or particulate matter (Singh et al., 2013).

Singh et al. (2013) recently revisited the distribution of N* over the North Atlantic and the estimated basin-scale yearly N\textsubscript{2} fixation rate. They corrected the value of N* considering the local variability of the nitrogen-to-phosphorus (N:P) ratio relative to the Redfield ratio of dissolved nutrients and particulate matter, as well as the deposition of atmospheric nitrogen. These authors argued that N* may have been significantly overestimated in previous publications by considering N:P = 16, when it is actually higher, for example, when *Trichodesmium* is abundant, or when there is an important deposition of high N:P atmospheric materials.

### 4.3. Contribution of N\textsubscript{2} fixation to P\textsubscript{new} and nitrogen excess

The exclusion of N\textsubscript{2} fixation rates as a source of P\textsubscript{new} can significantly underestimate the f-ratio (Raimbault and Garcia, 2008). N\textsubscript{2} fixation has been estimated to fuel up to 50% of primary production in oligotrophic oceanic areas (Capone et al., 2005). This was not the case in this study, where the contribution of N\textsubscript{2} fixation to P\textsubscript{new} was negligible (<1% in all cases). Similarly, Raimbault and Garcia (2008) did not find a significant contribution of N\textsubscript{2} fixation
to Pnew in the upwelling system off Chile. Benavides et al. (2011) measured gross and net N\textsubscript{2} fixation as derived from the acetylene reduction assay and the uptake of \textsuperscript{15}N\textsubscript{2}, respectively.

The difference between these two measurements is a good proxy for dissolved nitrogen release (Gallon et al., 2002). In the CAIBEX cruises, an average of \textasciitilde 60\% of the N\textsubscript{2} fixed was ‘lost’ as dissolved nitrogen (Benavides et al., 2011). Even if a 60\% increase is applied to all N\textsubscript{2} fixation rates, their contribution to Pnew is still <1\%. Mouriño-Carballido et al. (2011) compared N\textsubscript{2} fixation and NO\textsubscript{3}\textsuperscript{−} diffusion from 16ºN to 31ºS over central longitudes of the Atlantic. They found that, in the subtropical North Atlantic (\textasciitilde 16ºN–9ºN), N\textsubscript{2} fixation only contributed 2\% to Pnew.

N\textsubscript{2} fixation rates in our study area are likely affected by wind and coastal upwelling intensity, which are more intense in the summer off NW Iberia, but remain more constant throughout the year off NW Africa (Arístegui et al., 2009). Coastal upwelling provides high NO\textsubscript{3}\textsuperscript{−} concentrations, which raise Pnew rates (see the average NO\textsubscript{3}\textsuperscript{−} uptake and Pnew rates for the upwelling-affected area in Table 3). We must note that the measurements presented here correspond to the summer, when the upwelling intensity is maximum off Cape Ghir (Arístegui et al., 2009). Other factor potentially affecting our N\textsubscript{2} fixation rates is the deposition of atmospheric dust, brought by the nearby Saharan desert. Saharan dust contains iron and phosphorus, which are thought to be limiting for diazotrophic organisms (Mills et al., 2004). Nevertheless, dust inputs may not raise N\textsubscript{2} fixation rates significantly due to (1) a low bioavailability of Fe, for example in the absence of organic ligands; (2) dust particles sinking out of the photic zone before they can be utilized by diazotrophic organisms (Croot et al.,
2004); or (3) a less severe limitation by iron availability. Recently, Luo et al. (2013) gathered all the N₂ fixation data available in the literature and conducted statistical analyses to search for connections between the rates and a variety of environmental variables such as temperature, light and iron made available through dust deposition. Their results suggest that, on a global scale, iron does not play an important role in shaping the magnitude and distribution of N₂ fixation rates. Nevertheless, in general, in situ N₂ fixation measurements have shown that rates are boosted when a dust deposition event occurs (e.g. Benavides et al., 2013). Therefore, the role of iron in controlling N₂ fixation activity remains unclear and deserves further study. Moreover, it should be noted that the N₂ fixation rates presented are likely underestimated according to recent improvements of the ¹⁵N₂ method. N₂ fixation rates measured using ¹⁵N₂ bubble injections gives rates 50 to 570% times lower than when ¹⁵N₂ is added dissolved in sample seawater (Mohr et al., 2010; Großkopf et al., 2012), so a general increase of basin-scale N₂ fixation rates in the North Atlantic is expectable when the new method is applied in different locations.

As previously mentioned, Álvarez and Álvarez-Salgado (2007) estimated a net nitrogen excess accumulation rate for the area bounded between 25°– 42°N and 20°W of 40 ± 22 × 10¹⁰ mol N y⁻¹ or 658 ± 354 µmol N m⁻² d⁻¹ with a geochemical box model, which they hypothesized should be due to N₂ fixation after discounting other possible sources. This contrasts with the low average N₂ fixation rate measured in this study. Considering an average mixed layer depth of 100 m, the resulting volumetric rate of 6.6 ± 3.5 nmol N L⁻¹ d⁻¹ estimated from Álvarez and Álvarez-Salgado (2007) is still three orders of magnitude higher
the average rate measured experimentally in the same area (1.3 × 10⁻³ nmol N L⁻¹ d⁻¹; Benavides et al., 2011). If the average N₂ fixation rate for the CAIBOX area was 50% higher, the resulting average N₂ fixation rate of 2.4 × 10⁻³ nmol N L⁻¹ d⁻¹ would be still three orders of magnitude lower than estimated by Álvarez and Álvarez-Salgado (2007). Even if the highest increment observed between methods is applied (570% by Großkopf et al., 2012), the nitrogen excess rate observed would still be two orders of magnitude higher. Nevertheless, we must note that surface N₂ fixation rates are not necessarily representative of the whole water column. For example, the widespread unicellular diazotrophic cyanobacteria of group A (UCYN-A) are known to inhabit deeper layers of the world oceans (Moisander et al., 2010), and are the most abundant oceanic diazotrophs (Luo et al., 2012). Moreover, non-cyanobacterial diazotrophs such as α- or β-proteobacteria and Archaea also fix N₂ in deeper layers (Riemann et al., 2010). However, deep profiles of N₂ fixation rates in this area of the North Atlantic are not available in the literature to date. In summary, a better assessment of depth-integrated N₂ fixation rates in this area are needed in order to estimate its true contribution to Pnew.

4.4. Other sources of excess nitrogen accumulation in the water column

Nitrogen anomalies are caused by processes that happen in non-Redfieldian proportions, that is, processes that selectively accumulate nitrogen or selectively consume phosphorus (Landolfi et al., 2008). The uptake and regeneration of NO₃⁻ and NH₄⁺ are expected to occur in Redfieldian proportions; therefore, the nitrogen excess computed by
Álvarez and Álvarez-Salgado (2007) in our study area must be caused by other processes. Besides N_2 fixation, Landolfi et al. (2008) considered the following reasons behind nitrogen anomalies: (1) deposition of atmospheric combined nitrogen, which usually has high N:P ratios (Chen et al., 2007; Baker et al., 2010; Kanakidou et al., 2012); (2) advection and subsequent remineralization of phosphorus-depleted DOM (Hopkinson and Vallino 2005, Mather et al., 2008); and (3) preferential uptake and/or remineralization of phosphorus over nitrogen (Clark et al., 1998). Below, we include a brief discussion of the role of each of these possibilities in the CAIBOX area (non upwelling-affected stations, Figure 1a). Nitrogen excess estimations are not available for the upwelling-affected area (Cape Ghir, Figure 1b), and therefore will not be further discussed here.

(1) The contribution of atmospheric combined nitrogen deposition to the nitrogen anomaly in the North Atlantic has been previously argued (e.g. Landolfi et al., 2008; Zamora et al., 2010). However, as discussed in Álvarez and Álvarez-Salgado (2007), present modeled rates of atmospheric combined nitrogen deposition to the North Atlantic (compiled in Table 5) do not explain the nitrogen excess observed in the CAIBOX area.

(2) A second factor behind nitrogen anomalies would be the injection of dissolved organic matter (DOM) with an N:P ratio > 16 into the mesopelagic layer before it is fully remineralized (Mahaffey et al., 2005; Hansell et al., 2007). It is important to note that the CAIBOX area is bounded to the east by the coastal upwelling system of Iberia – NW Africa. In this sense, García-Muñoz et al. (2005), observed that ~60% of the net primary production near Cape Ghir (~30–31°N) was exported as DOM via upwelling filaments. These are
recurrent features of the Iberian-NW African upwelling system and may extend hundreds of kilometers offshore, impacting the thermocline of the North Atlantic subtropical gyre (Álvarez-Salgado et al., 2007). In addition, the release of DON derived from NO$_3^-$ and NH$_4^+$ uptake represented considerable percentages of gross uptake (Table 4). This suggests that, adding to atmospheric combined nitrogen deposition, DON advected from the coastal upwelling, and DON produced in situ through NO$_3^-$ and NH$_4^+$ uptake may contribute substantially to the observed nitrogen excess in this area.

(3) In contrast with the DON pool, which contains semilabile and refractory fractions (Bronk et al., 2007), the dissolved organic phosphorus (DOP) pool is mostly biologically available thanks to the widespread alkaline phosphatase enzyme (Dyhrman et al., 2007). Low inorganic phosphorus availability in the North Atlantic triggers the uptake of DOP, which is selectively remineralized over DON (Kolowith et al., 2001). The preferential remineralization of phosphorus over nitrogen should be assessed by the observation of profiles of DON:DOP ratios (Clark et al., 1998), but unfortunately, DOP data are not available for this study.

4.5. Nitrogen budgets in the CAIBOX area

Álvarez and Álvarez-Salgado (2007) (Figure 5a) assumed that the organic nitrogen concentrations measured by Doval et al. (2001) in the eastern North Atlantic–Azores Front region were representative for the whole study area to calculate the organic nitrogen flux from the adjacent ocean into the CAIBOX area. Within the framework of the CAIBEX project, full-depth organic nitrogen profiles were measured in the CAIBOX area in such a
way that organic nitrogen fluxes can be recalculated more precisely. Following Álvarez and Álvarez-Salgado (2007), the total transport of organic nitrogen from the open ocean to the CAIBOX area can be ascribed to the Ekman (0.25 \times 10^6 \text{ m}^3 \text{ s}^{-1}) and overturning (3 \times 10^6 \text{ m}^3 \text{ s}^{-1}) transports. The average total organic nitrogen concentration in the surface layer of the CAIBOX area was 6.7 \pm 1.0 \mu \text{M}, which translates into an organic nitrogen flux into the CAIBOX of 5.4 \pm 0.8 \times 10^{10} \text{ mol N y}^{-1} due to Ekman transport. The average total organic nitrogen concentration in the upper 200 m and from 500 m to the sea-bottom of the CAIBOX area were 6.4 \pm 1.0 and 3.9 \pm 1.0 \mu \text{M}. The vertical gradient, estimated as the difference between the mean concentration below 500 m and that in the upper 200 m, multiplied by the overturning transport, yielded an organic nitrogen flux into the CAIBOX of 26 \pm 13 \times 10^{10} \text{ mol N y}^{-1}. Therefore, the recalculated organic nitrogen flux into the box is 32 \pm 13 \times 10^{10} \text{ mol N y}^{-1} (Figure 5c). The previous estimate of the flux of NO$_3^-$ from the Mediterranean into the Atlantic Ocean was 27 \pm 13 \times 10^{10} \text{ mol N y}^{-1} (Figure 5b), which is considerably reduced considering the more recent estimate of 13.9 \pm 0.3 \times 10^{10} \text{ mol N y}^{-1} (Figure 5d) provided by Huertas et al. (2012).

Furthermore, Álvarez and Álvarez-Salgado (2007) did not account for the previously referred export of organic nitrogen from the coastal upwelling of Iberia–NW Africa, which amounted of 19 \times 10^{10} \text{ mol y}^{-1}, because they considered that this coastal upwelling system was within the limits of their box model. Therefore, they assumed that the organic nitrogen produced in the coast, exported to the open ocean, and accumulated within the box, would be part of the recycled production of the system and would not contribute to the net production...
budget. However, it has been suggested (Hansell and Carlson 2002; Hansell et al., 2009) that
the fate of the material exported from the coast is to accumulate in the adjacent ocean; in this
case, the steady-state assumption of Álvarez and Álvarez-Salgado et al. (2007) would not be
accurate, implying that part (if not all) of the $19 \times 10^{10}$ mol N y$^{-1}$ of the nitrogen excess could
be explained by accumulation within the limits of the box (Figure 5c). In summary, if the
original budget by Álvarez and Álvarez-Salgado et al. (2007) is revisited incorporating new
flux estimates of the import of NO$_3^-$ from the Mediterranean (Huertas et al., 2012), the
organic nitrogen from the surrounding ocean and the accumulation of organic nitrogen from
the adjacent coastal upwelling system, the nitrogen excess would reduce to $22 \pm 19 \times 10^{10}$
mol N y$^{-1}$ or $353 \pm 306$ µmol N m$^{-2}$ d$^{-1}$. This nitrogen excess rate is about half of the
previous estimate by Álvarez and Álvarez-Salgado (2007). In conclusion, although the
revisited rate is lower than previously estimated, this excess is still not explained by in situ
measurements of N$_2$ fixation and estimates of atmospheric fixed nitrogen deposition. Further
research is therefore needed to constrain nitrogen inputs and outputs in this area of the eastern
North Atlantic.

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References


important source of new nitrogen to the tropical and subtropical North Atlantic Ocean.


Table 1: Equations used to determine $^{15}$N-labeled substrate uptake (nitrate, ammonium or dinitrogen -NO$_3^-$, NH$_4^+$ or N$_2$), regeneration and release as dissolved organic nitrogen (DON).

<table>
<thead>
<tr>
<th>$^{15}$N-labeled substrate</th>
<th>Equation</th>
<th>Description</th>
<th>Reference</th>
</tr>
</thead>
</table>
| $^{15}$NO$_3^-$ | $\rho_{NO3} \frac{R_{PON} \cdot PON}{R_{NO3} \cdot t}$ | $\rho_{NO3} = NO_3^-$ uptake  
$R_{PON} = PON$ excess $^{15}$N at.% enrichment  
$PON = $ final particulate organic nitrogen concentration  
$R_{NO3} = NO_3^- 15N$ at.% enrichment  
$t = $ incubation time | Dugdale and Wilkerson (1986) |
| $^{15}$NO$_3^-$ | $DONr_{NO3} = \frac{R_{DON} \cdot DON}{R_{NO3} \cdot t}$ | $DONr_{NO3} = DON$ release from NO$_3^-$ uptake  
$R_{DON} = DON$ excess $^{15}$N at.% enrichment  
$DON = $ final DON concentration  
$R_{NO3} = NO_3^- 15N$ at.% enrichment  
$t = $ incubation time | Slawyk et al. (1998) |
| $^{15}$NH$_4^+$ | $\rho_{NH4} \frac{R_{PON} \cdot PON}{R_{NH4} \cdot t}$ | $\rho_{NH4} = NH_4^+$ uptake  
$R_{PON} = PON$ excess $^{15}$N at.% enrichment  
$PON = $ final particulate organic nitrogen concentration  
$R_{NH4} = NH_4^+ 15N$ at.% enrichment  
$t = $ incubation time  
$k = $ dilution factor  
$R_{NH4i} = NH_4^+ 15N$ at.% enrichment  
$R_{NH4f} = NH_4^+ 15N$ at.% enrichment | Glibert et al. (1982) |
| $^{15}$NH$_4^+$ | $r_{NO3} = \frac{R_{NO3} \cdot NO3}{R_{NH4} \cdot t}$ | $r_{NO3} = NO_3^-$ regeneration  
$NO3 = $ final NO$_3^-$ concentration  
$R_{NH4} = final NH_4^+ 15N$ at.% enrichment  
$t = $ incubation time | Fernández and Raimbault (2007) |
| $^{15}$NH$_4^+$ | $r_{NH4} = \frac{NH4_i + NH4_f}{2 \cdot t} \cdot \ln \left( \frac{R_{NH4f}}{R_{NH4i}} \right)$ | $r_{NH4} = NH_4^+$ regeneration  
$NH4_i = initial NH_4^+$ concentration  
$NH4_f = final NH_4^+$ concentration  
$t = $ incubation time  
$R_{NH4i} = NH_4^+ 15N$ at.% enrichment  
$R_{NH4f} = NH_4^+ 15N$ at.% enrichment | Fernández and Raimbault (2007) |
| $^{15}$NH$_4^+$ | $DONr_{NH4} = \frac{R_{DON} \cdot DON}{R_{NH4} \cdot t}$ | $DONr_{NH4} = DON$ release from NH$_4^+$ uptake  
$R_{DON} = DON$ excess $^{15}$N at.% enrichment  
$DON = $ final DON concentration  
$R_{NH4} = NH_4^+ 15N$ at.% enrichment  
$t = $ incubation time | Slawyk et al. (1998) |
| $^{15}$N$_2$ | $\rho_{N2} \frac{R_{PON} \cdot PON}{R_{N2} \cdot t}$ | $\rho_{N2} = N_2$ uptake (N$_2$ fixation)  
$R_{PON} = PON$ excess $^{15}$N at.% enrichment  
$PON = $ final particulate organic nitrogen concentration  
$R_{N2} = N_2 15N$ at.% enrichment  
$t = $ incubation time | Montoya et al. (1996) |
Table 2: Position of the mixed layer depth (MLD) and the deep chlorophyll maximum (DCM) at the stations sampled in the non upwelling-affected and upwelling-affected areas. In situ concentrations (previous to incubations with added $^{15}$NH$_4^+$ or $^{15}$NO$_3^-$) of nitrate (NO$_3^-$), ammonium (NH$_4^+$) at the surface and at the DCM of all the stations sampled. Under detection limit NH$_4^+$ concentrations are depicted as n/d (not detectable).

<table>
<thead>
<tr>
<th>Area</th>
<th>Station</th>
<th>Latitude (°N)</th>
<th>Longitude (°N)</th>
<th>MLD (m)</th>
<th>DCM (m)</th>
<th>Surface NO$_3^-$ (µM)</th>
<th>DCM NO$_3^-$ (µM)</th>
<th>Surface NH$_4^+$ (µM)</th>
<th>DCM NH$_4^+$ (µM)</th>
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<td>Non upwelling-affected</td>
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<td>10.58</td>
<td>40</td>
<td>60</td>
<td>0.13</td>
<td>0.12</td>
<td>–</td>
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<td>0.09</td>
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<td>20.01</td>
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<td>0.14</td>
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<td></td>
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<td>34.99</td>
<td>19.97</td>
<td>40</td>
<td>80</td>
<td>0.08</td>
<td>0.08</td>
<td>n/d</td>
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<tr>
<td></td>
<td>X12</td>
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<td>20.00</td>
<td>45</td>
<td>105</td>
<td>0.13</td>
<td>0.11</td>
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<td>29.85</td>
<td>19.19</td>
<td>45</td>
<td>90</td>
<td>0.17</td>
<td>0.18</td>
<td>n/d</td>
<td>0.019</td>
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<tr>
<td></td>
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<td>15.93</td>
<td>50</td>
<td>110</td>
<td>0.05</td>
<td>0.05</td>
<td>n/d</td>
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</tr>
<tr>
<td>Upwelling-affected</td>
<td>GM2</td>
<td>31.00</td>
<td>10.01</td>
<td>15</td>
<td>25</td>
<td>3.00</td>
<td>2.77</td>
<td>n/d</td>
<td>n/d</td>
</tr>
<tr>
<td></td>
<td>G12</td>
<td>30.74</td>
<td>10.65</td>
<td>15</td>
<td>30</td>
<td>0.61</td>
<td>1.95</td>
<td>n/d</td>
<td>0.009</td>
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<tr>
<td></td>
<td>G17</td>
<td>30.90</td>
<td>10.80</td>
<td>20</td>
<td>40</td>
<td>1.35</td>
<td>2.20</td>
<td>0.002</td>
<td>0.072</td>
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<tr>
<td></td>
<td>G22</td>
<td>31.00</td>
<td>10.91</td>
<td>25</td>
<td>40</td>
<td>0.38</td>
<td>1.56</td>
<td>0.015</td>
<td>0.305</td>
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<tr>
<td></td>
<td>GT2</td>
<td>31.11</td>
<td>10.60</td>
<td>25</td>
<td>45</td>
<td>0.82</td>
<td>0.93</td>
<td>n/d</td>
<td>n/d</td>
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<tr>
<td></td>
<td>G40</td>
<td>31.01</td>
<td>11.31</td>
<td>30</td>
<td>48</td>
<td>0.55</td>
<td>1.65</td>
<td>n/d</td>
<td>n/d</td>
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<tr>
<td></td>
<td>G44</td>
<td>30.95</td>
<td>11.40</td>
<td>28</td>
<td>43</td>
<td>1.50</td>
<td>0.38</td>
<td>n/d</td>
<td>0.021</td>
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<tr>
<td></td>
<td>G48</td>
<td>30.89</td>
<td>11.45</td>
<td>27</td>
<td>50</td>
<td>0.76</td>
<td>1.46</td>
<td>0.007</td>
<td>n/d</td>
</tr>
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</table>
Table 3: Average and standard deviation (± SD) values of NO$_3^-$ and NH$_4^+$ uptake and regeneration, and dissolved organic nitrogen (DON) release derived from NO$_3^-$ uptake (DON-NO$_3^-$) and from NH$_4^+$ uptake (DON-NH$_4^+$), and N$_2$ fixation, all in µmol L$^{-1}$ h$^{-1}$. All values are average measurements made at the surface (5 m) and at the deep chlorophyll maximum (DCM), except for N$_2$ fixation, which was only measured at the surface. NO$_3^-$ regeneration was not detectable (n/d) in the upwelling-affected stations.

<table>
<thead>
<tr>
<th></th>
<th>NO$_3^-$ uptake</th>
<th>NH$_4^+$ uptake</th>
<th>NO$_3^-$ regeneration</th>
<th>NH$_4^+$ regeneration</th>
<th>DON-NO$_3^-$ release</th>
<th>DON-NH$_4^+$ release</th>
<th>N$_2$ fixation</th>
<th>Pnew</th>
<th>Preg</th>
<th>f-ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non upwelling-affected</td>
<td>0.008 ± 0.007</td>
<td>0.143 ±0.187</td>
<td>0.002 ± 0.002</td>
<td>0.012 ± 0.013</td>
<td>0.008 ± 0.486</td>
<td>0.001 ± 0.002</td>
<td>1.3 ± 0.7 x10$^{-6}$</td>
<td>0.014</td>
<td>0.157</td>
<td>0.084</td>
</tr>
<tr>
<td>Upwelling affected</td>
<td>0.122 ± 0.086</td>
<td>0.105 ± 0.078</td>
<td>n/d</td>
<td>0.008 ± 0.023</td>
<td>0.004 ± 0.006</td>
<td>0.020 ± 0.033</td>
<td>5 ± 5 x10$^{-6}$</td>
<td>0.126</td>
<td>0.133</td>
<td>0.487</td>
</tr>
</tbody>
</table>
Table 4: Percentage DON release contribution to gross $\text{NO}_3^-$ and $\text{NH}_4^+$ uptake in non upwelling-affected and upwelling-affected areas.

<table>
<thead>
<tr>
<th>Area</th>
<th>DON release from $\text{NO}_3^-$ contribution to gross $\text{NO}_3^-$ uptake (%)</th>
<th>DON release from $\text{NH}_4^+$ contribution to gross $\text{NH}_4^+$ uptake (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non upwelling-affected</td>
<td>26.48 ± 30.41</td>
<td>2.21 ± 3.18</td>
</tr>
<tr>
<td>Upwelling-affected</td>
<td>2.59 ± 2.67</td>
<td>14.16 ± 13</td>
</tr>
</tbody>
</table>
Table 5: Modeled rates of atmospheric nitrogen deposition to the North Atlantic compiled in Baker et al. (2010). Deposition rates were recalculated for the CAIBOX area considering a surface equal to $1.1 \times 10^{12}$ m$^2$.

<table>
<thead>
<tr>
<th>Reference</th>
<th>North Atlantic atmospheric nitrogen deposition (Gmol N y$^{-1}$)</th>
<th>CAIBOX atmospheric nitrogen deposition (mol N y$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duce et al. 1991</td>
<td>619</td>
<td>1.27 x 10$^{10}$</td>
</tr>
<tr>
<td>Prospero et al. 1996</td>
<td>409</td>
<td>0.84 x 10$^{10}$</td>
</tr>
<tr>
<td>Dentener et al. 2006</td>
<td>492</td>
<td>1.01 x 10$^{10}$</td>
</tr>
<tr>
<td>Luo et al. 2007</td>
<td>462</td>
<td>0.95 x 10$^{10}$</td>
</tr>
<tr>
<td>Baker et al. 2010</td>
<td>483</td>
<td>0.99 x 10$^{10}$</td>
</tr>
<tr>
<td>Baker et al. 2010</td>
<td>456–710</td>
<td>*1.12–1.46 x 10$^{10}$</td>
</tr>
</tbody>
</table>

*Considering a 13–47% increase in nitrogen deposition rates when soluble organic nitrogen is added.