The importance of nitrogen fixation to the nitrogen budget of the North Atlantic Ocean.

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SUMMARY

Samples of seawater and suspended particles in the mixed layer (top 100m) were collected on *Seward Johnson* cruises SJ9603, SJ9612, and SJ0005 to the tropical and subtropical North Atlantic Ocean. Deep-water nitrate has been proposed to provide most nitrogen to the upper water column of the North Atlantic Ocean. Recent evidence has shown that N_2 fixation is plays a significant role in supplying nitrogen for new production. The ratio of ^{15}N: ^{14}N, referred to as $\delta^{15}N$, provides a useful tracer for identifying major sources to new nitrogen in the upper water column. Persistently low $\delta^{15}N$ values coupled with high N* values imply a large contribution of N_2 fixation, with 83 of 85 stations suggesting some contribution of N_2 fixation. The highest levels of diazotrophic contribution were recorded in the southwestern tropical Atlantic Ocean, where a large bloom of the N_2 fixing diatom/ cyanobacterial association *Hemiaulus/ Richelia* association in addition to the N_2 fixing cyanobacterium *Trichodesmium* was previously recorded. The isotopic data, N* data, and diazotrophic contribution estimates show N_2 fixation is making a significant contribution to the nitrogen budget of the nutrient-poor North Atlantic Ocean.

CHAPTER 1

INTRODUCTION

The Marine Nitrogen Cycle

Recent studies have proven that the marine nitrogen cycle is much more dynamic than previously estimated. In previous studies, small sampling times and areas have left large and imprecise ranges for processes contributing to the nitrogen cycle (Gruber and Sarmieneto 1997). The marine nitrogen cycle is also closely linked to the carbon cycle through the biological pump (Michaels *et al.* 2001). The balance between inputs of new nitrogen and exports of nitrogen through sinking particles, vertical migration, and mixing is responsible for the sequestration of atmospheric carbon in the deep- sea and therefore, the function of the biological pump (Hansell *et al.* 2004). Because nitrogen is an important structural component of cells and is thought to be the limiting nutrient in many marine ecosystems, it can provide a useful parameter to measure ecosystem production (Dugdale and Goering 1967). As marine nitrogen is removed through denitrification, new inputs of nitrogen are needed to support marine communities (Hansell *et al.* 2004, Dugdale and Goering 1967). The fraction of net primary production that is supported by external sources of nitrogen will herein after be referred to as new nitrogen. However, identifying the sources of new marine nitrogen and quantifying their contributions has proved difficult and controversial.

Upwelling of deep-water nitrate was traditionally thought to be the main source of new nitrogen supporting primary production (Capone *et al.* 2005, Montoya *et al*. 2002). The amount of nitrogen needed to support new production in the ocean greatly exceeds the

amount estimated to be available by upwelling of deep-water nitrate (Capone *et al.* 2005, Reynolds *et al.* 2007). This presents a problem, as the amount of nitrogen exported must be in balance with the amount of new nitrogen available if phytoplankton communities are to persist (Dugdale and Goering 1967). The process of reducing N_2 to the biologically more useful, combined nitrogen form of ammonia is referred to as N_2 fixation (Giller 2001). In recent years, evidence has been building that biological N_2 fixation has been greatly underestimated as a source of new nitrogen supporting new production (Carpenter *et al.* 1999, Dugdale and Goering 1967, Laroche and Breitbarth 2005, Michaels *et al.* 2001). Colonial cyanobacteria in the genus *Trichodesmium* have been widely considered the most significant oceanic N_2 fixers (Laroche and Breitbarth 2005, Mulholland 2006). However, diatoms with heterocystous symbionts and, more recently, unicellular diazotrophs have also been shown to make large contributions to new production in oligotrophic, or nutrient poor, waters (Carpenter *et al.* 1999, Montoya *et al.* 2004, LaRoche and Breitbarth 2005). N_2 fixation is thought to be especially important to sustaining phytoplankton populations in oligotrophic waters that would otherwise be bankrupt of nitrogen (Mahaffey *et al.* 2003). Poorly characterized global distribution and bloom characteristics, in addition to large discrepancies between biological and geochemical estimates of N_2 fixation rates have resulted in uncertainty in the scientific community as to the role that N_2 fixation is playing in the world's oceans (Laroche and Breitbarth 2005, Hansell *et al.* 2004).

Approach

The ratio of ^{15}N: ^{14}N, represented by $\delta^{15}N$, can provide a way of identifying nitrogen sources that support marine plankton communities (Reynolds *et al*. 2007 Montoya *et al.* 2002). The mean $\delta^{15}N$ of deep-water nitrate is around 4.5 ‰, while the $\delta^{15}N$ of nitrogen

produced by N_2 fixation has a much lower isotopic signal of -2 ‰ (Liu and Kaplan 1989, Reynolds *et al.* 2007). Due to this difference in characteristic $\delta^{15}N$ values, examining isotopic nitrogen signals of suspended particles in the North Atlantic can identify the major sources supplying nitrogen to this nutrient poor area, and their contribution to new production can be assessed.

While depleted $\delta^{15}N$ signals are important in the implication of marine N_2 fixation, they are not unique to N_2 fixation and can be explained by several marine processes. One possible explanation of depleted signals is the preferential export of ^{15}N out of the upper water column through food web processes (Montoya *et al.* 2002). Food web processes could contribute to depleted $\delta^{15}N$ values by reserving ^{14}N in the surface layer as recycled NH_4^+, while ^{15}N is removed from the mixed layer in sinking fecal pellets (Montoya *et al.* 2002). Another possibility is atmospheric deposition of isotopically light N, but this seems to be relatively unimportant to the North Atlantic Ocean (Reynolds *et al.* 2007). Also, as previously stated, direct fixation of dissolved N_2 will produce isotopically depleted nitrogen, which can give rise to depleted $\delta^{15}N$ values (Liu and Kaplan 1989, Reynolds *et al.* 2007). In order to distinguish between these processes, we can utilize changes in nutrient ratios through the quasi-conservative tracer N*. This tracer serves to investigate the distribution and importance of N_2 fixation through a linear combination of nitrate and phosphate (Gruber and Sarmiento 1997). Specifically, high concentrations of N* correspond to elevated N: P ratios, indicating inputs of new nitrogen from N_2 fixation. (Gruber and Sarmiento 1997, Mahaffey *et al.* 2003) This results from the fact that inputs of nitrogen from nitrogen fixation will cause the ratio of N:P to deviate from Redfield stoichiometry in favor of nitrogen. Measuring the location and severity of these deviations can identify areas where

N_2 fixation is especially important and can determine how influential N_2 fixation is in the region. Coupling N* data with isotopic analysis will give a robust analysis of the role that N_2 fixation is playing in supporting the communities of the tropical and subtropical North Atlantic Ocean.

We will use isotopic analysis and nutrient ratios to identify important nutrient sources, and determine the role that diazotrophic N_2 fixation is playing in supporting ecosystems in the oligotrophic North Atlantic. This will be done through measuring $\delta^{15}N$ values for suspended particles, using a mixing model to quantify the diazotrophic contribution from N_2 fixation, and measuring N* values.

CHAPTER 2

METHODS

Isotopic Analysis

The methods used for isotopic analysis follow the procedure of *Montoya et al.* (2002), and are briefly described below.

Samples of seawater and suspended particles in the upper water column (top 100m) were collected from 85 stations and analyzed for comparison of ^{15}N: ^{14}N ratios. These samples were obtained on *Seward Johnson* cruises SJ9603, SJ9612, and SJ0005 to the tropical and subtropical North Atlantic Ocean in April 1996, October 1996 and April- May 2000, respectively (Figure 1). Samples were gathered by a conductivity-temperature-depth, or CTD rosette system. Suspended particles were collected from gentle vacuum filtration of water samples (200 mm Hg vacuum) through 45 mm GF/F filter, precombusted at 450 C for 2 hours. Between 4L and 8L of seawater was filtered, then each filter was dried at 60 C for on shore analysis. Once ashore, filters were trimmed and then cut into eighths, quadrants, or halves depending on the particle density. Each filter section was subsequently pelletized in tin capsules for isotopic analysis.

Continuous flow isotope ratio mass spectrometry was used for isotopic and natural abundance measurements of the cruise samples. Three different machines were used: a VG Prism II at Harvard, a Europa 20-20 at the Chesapeake Biological Laboratory, or a Micromass Optima at the Georgia Institute of Technology. The three machines were intercalibrated by running various organic and inorganic standards on each machine, and also by running portions of the same sample on multiple machines. All $\delta^{15}N$ values are

expressed with respect to atmospheric N_2. Each sample run included a size series of isotopic and elemental standards in an effort to check the reliability of the data. We can estimate that our isotopic measurements are precise within ±0.03%.

Assessing the Role of N_2 Fixation

The percent of Nitrogen supplied by N_2 fixation was calculated based on an isopycnal mixing model utilizing the weighted mean of $\delta^{15}N$ at each station. The mean $\delta^{15}N$ value for each station was weighted to give $\delta^{15}N$ values from higher concentrations of N in the water column more importance in the station mean than $\delta^{15}N$ values from low concentrations. For our purposes, this weighted mean should give a more accurate look into the processes supplying new production. The isopycnal mixing model is expected to provide a conservative estimate of N_2 fixation while minimizing possible discrepancies between biological and geochemical estimates of N_2 fixation (Hansell et al. 2004). We used formula 1 below to estimate the importance of diazotrophic inputs to the suspended particles in the mixed layer:

$$\% \text{ Diazotrophic N} = 100 * \left(\frac{\partial^{15}N_{particles} - \partial^{15}NO_3^-}{\partial^{15}N_{diazotroph} - \partial^{15}NO_3^-} \right) \qquad (1)$$

where we use $\delta^{15}N0_3^-$ to be 4.5 ‰ and $\delta^{15}N_{\text{ dizaotroph}}$ to be -2 ‰.

N* values were calculated according to formula 2, originally described by *Gruber and Sarmiento 1997.*

$$N^* = (N - 16P + 2.90 \quad \text{mol kg}^{-1}) \; 0.87 \qquad (2)$$

Values of N* were plotted on the isopycnal surface $Sigma_T$ [in situ Density (z,T,s)] = 26.7.

CHAPTER 3

RESULTS

Excluding station 2 of SJ9612 (72.509 W, 27.050 N) which shows a high $\delta^{15}N$ of 9.89‰, the $\delta^{15}N$ for the other 84 stations falls in the range of -2.28 ‰ and 4.64 ‰, with a mean of 1.61 ‰± 0.18 (mean ± SE, n= 85). This mean $\delta^{15}N$ value corresponds to a 44.5% contribution of nitrogen fixation. A histogram of $\delta^{15}N$ shows these values are consistently low, implying consistent inputs of nitrogen from N_2 fixation in the North Atlantic (Figure 2B).

Values for the contribution of N_2 fixation showed the greatest range in the SJ9612 data set, with one station, station 8 (54.872 W, 16.849 N), suggesting 100% of nitrogen in the upper water column supplied by N_2 fixation, and another station, station 2 (72.508 W, 27.049 N), suggesting no contribution (Figure 3A, Figure 3B). Station 2 of SJ9612 which reported the elevated $\delta^{15}N$ of 9.89 ‰ was one of only two stations suggesting no diazotrophic contribution, the other being station 19 (29.817 W, 16.283 N) of SJ9603 with a $\delta^{15}N$ of 4.64 ‰.

There is a general negative correlation between diazotrophic contribution and latitude (Figure 3B). The leg of SJ0005 extending laterally across the basin from 75 to 21.2 W at approximately 32 N (Figure 4) shows no such correlation with longitude. The $\delta^{15}N$ values for this trans-basin section range from 1.04 ‰ to 4.16 ‰, with a mean of 2.20 ‰ ± 0.16 (mean ± SE, n=29). This range corresponds to between 53.23% and 5.23% contribution of N_2 fixation to this leg of SJ0005. The N* values for this leg mirror the

results from the $\delta^{15}N$ analysis, reporting high values of above 2 mol kg^{-1} for every station except station 44 (35 W, 32.37 N) which has an N* value of 0.44 mol kg^{-1} (Figure 5). South of this leg in the southwestern tropical North Atlantic, nitrogen isotopic levels report their lowest values, and the contribution of N_2 fixation increases.

The samples originating in the southwestern tropical North Atlantic have a range of $\delta^{15}N$ between -2.283 ‰ and 3.678 ‰ with a mean of 0.59 ‰ ± .23 (mean ± SE, n=29). The corresponding range for the diazotrophic contribution of N_2 fixation is between 100% and 12.65%, with a mean of 60.15%. The stations extending into the middle of the basin reported a range of $\delta^{15}N$ values between -0.792 ‰ and 4.643 ‰ with a mean value of 1.997‰. ± 0.35 (mean ± SE, n=14), which corresponds to a contribution of N_2 fixation between 81.41% and 0%, with a mean contribution of 38.51%. The northernmost samples reported a $\delta^{15}N$ range between -0.093 ‰ and 9. 86 ‰, which translates to a diazotrophic contribution in the range of 70.66% to 0% .The mean $\delta^{15}N$ value in this region is 2.32 ‰ ± .25 (mean ± SE, n=42), or a 33.56% contribution of N_2 fixation.

The southwestern region of the basin also reported high N* anomalies, (Figure 5) complementing the low $\delta^{15}N$ values and high contribution of N_2 fixation in this region. The local maximum of N* of 11.22 mol kg^{-1} is located at SJ9612 station 14 (44.086 W, 8.617 N) which possesses a depleted $\delta^{15}N$ value of 0.280 ‰. Stations between 0 and 10 N and 50 and 40 W show consistently high concentrations of N*. While the southwestern region reports the highest values of N*, the stations extending into the middle of the basin also show very high values, supporting the low $\delta^{15}N$ values previously reported for this region. Overall, the N* values roughly coincide with the results from the isotopic analysis, confirming N_2 as an important process supplying new nitrogen to this region.

CHAPTER 4

DISCUSSION

We are addressing the question of the significance of N_2 fixation to the overall nitrogen budget of the North Atlantic Ocean. The results from the $\delta^{15}N$ analysis reveal large implications of N_2 fixation to the system through consistently low $\delta^{15}N$ values. While the range of $\delta^{15}N$ values is large (-2.28 ‰ - 9.89 ‰), the contribution of N_2 fixation is obvious through the majority of low $\delta^{15}N$ values (Figure 2B). In fact, 83 out of 85 stations fall below the $\delta^{15}N$ value of deep-water nitrate, implying another, isotopically lighter nitrogen source. These low values cannot be explained by the upwelling of deep-water nitrate (Figure 2B), and are confirmed by the high concentrations of N* (Figure 5). Nitrogen produced by N_2 fixation is isotopically depleted with signals of around -2 ‰, which suggests that N_2 fixation is playing a significant role in supplying new nitrogen across the North Atlantic Ocean. The mean value of $\delta^{15}N$ reported from these studies is 1.61‰ ± 0.18 (mean ± SE, n= 85), too low to be explained by upwelling alone. If nitrogen supporting primary production were supplied by upwelling of deep- water nitrate, the mean $\delta^{15}N$ presumably would be higher and closer to 4.5 ‰. Therefore, the persistently low $\delta^{15}N$ values across the North Atlantic Ocean can be interpreted as a result of large inputs of nitrogen from N_2 fixation. This interpretation is justified by the accompanying high values of N*, which distinguish N_2 fixation as the source of the depleted isotopic signals over other explanations such as food web processes.

While the isotopic data, N* values, and the contribution calculations suggest that N_2 fixation is present and important to the middle and northern areas of the basin, N_2 fixation

appear to be most influential in the southwestern region. In the southwestern tropical Atlantic Ocean where our data shows a large contribution of diazotrophic N_2 fixation (Figure 3A), strong positive N* anomalies have been observed in previous studies (Capone *et al.* 2005, Gruber and Sarmiento 1997, Hansell *et al.* 2004). Our data confirms this, as we observed constantly elevated N* concentrations in addition to the local maximum in the southwestern region (Figure 5). These persevering high N* values solidify the significance of the low $\delta^{15}N$ values and confirm the importance of N_2 fixation to the southwestern tropical Atlantic Ocean.

Furthermore, *Carpenter et al.* (1999) reported a large bloom of the *Hemiaulus/ Richelia* association in this same region in autumn of 1996, when the data for this study was being collected. While this association is frequently found in tropical regions, the 1996 bloom was attributed to plumes of the Amazon River and Orinoco River (Carpenter *et al.* 1999). *Carpenter et al.* (1999) also reported the N_2 fixing cyanobacteria *Trichodesmium* present at several stations throughout the bloom. The bloom showed exceptionally high rates of N_2 fixation, and isotopic data observed in the food web suggested the bloom had persisted for a significant amount of time (Carpenter *et al.* 1999). This high density of N_2 fixers most likely contributed to the low isotopic signal in the southwestern tropical Atlantic Ocean, and the significant negative correlation between diazotrophic contribution and latitude. The isotopic data, along with the estimates of diazotrophic contribution show high inputs of isotopically depleted nitrogen produced by N_2 fixation to the North Atlantic Ocean. While N_2 fixation appears to be important over a vast area, our data suggest that it is especially important to in the southwestern area of the tropical North Atlantic Ocean. Depleted measurements of $\delta^{15}N$, N* measurements, and calculations for the contribution of

diazotrophic N_2 fixation collectively provide very strong support for the importance of N_2 fixation to the nitrogen budget of the oligotrophic North Atlantic.

APPENDIX A

FIGURES

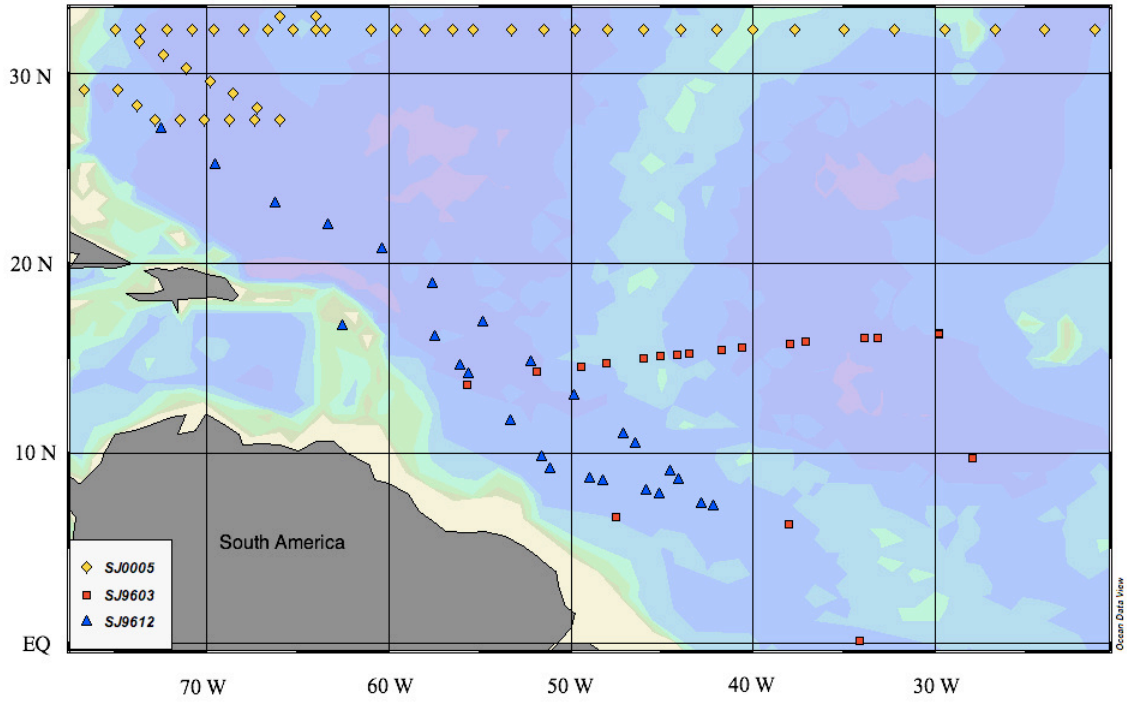

Figure 1*. Cruise tracks for SJ9603 (April 1996), SJ9612 (October 1996), and SJ0005 (April- May 2000). SJ0005, represented by yellow diamonds, provides a cross-basin look at the subtropical North Atlantic Ocean at approxiamtely 32 N. SJ9612, represented by blue triangles, provided sampling in the southwestern tropical region of the basin. SJ9603, represented by red squares, extended south roughly along the coast of South America before travelling into the middle of the basin. From the middle of the basin, SJ9603 returns westward at approximately 14 - 16 N, connecting the samples from the southwest to samples from the interior.*

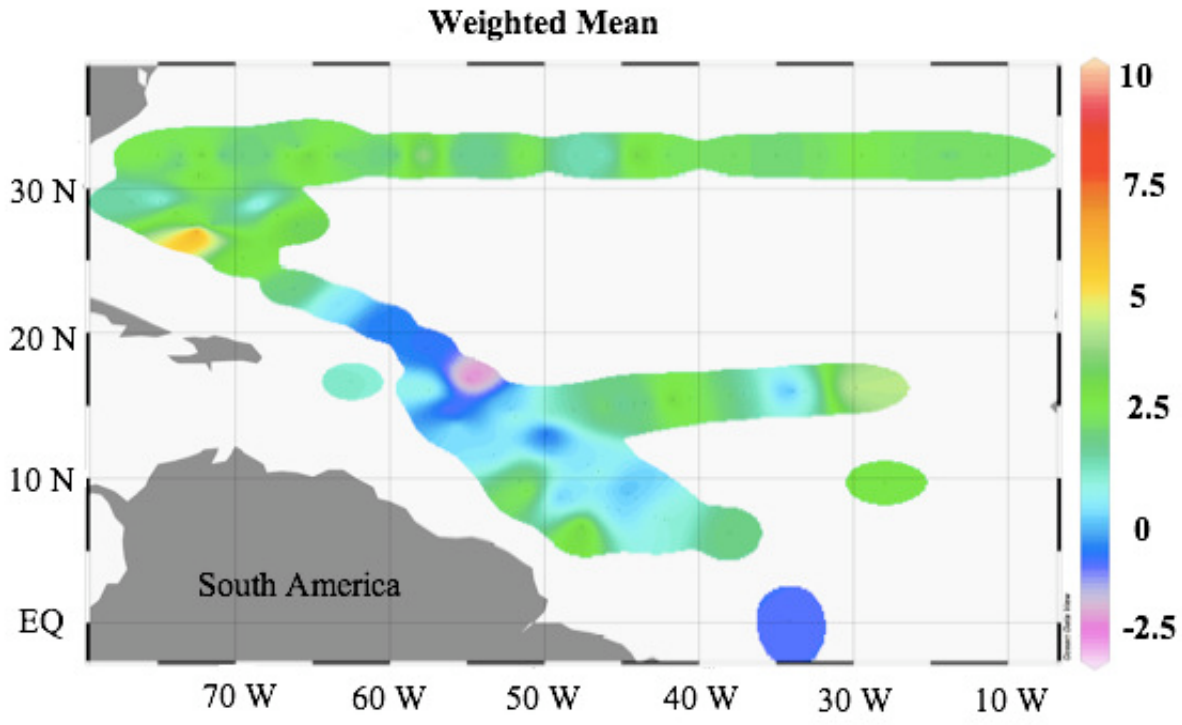

Figure 2A. *Geographic distribution of the weighted mean of $\delta^{15}N$. White represents area not sampled on the cruises. Local maximum weighted mean is located at station 2 of SJ9612 (72.508 W, 27.049 N) with a value of 9.89‰. Local minimum shown at SJ9612 station 8 (54.872 W, 16.849 N) with a value of -2.28‰. Weighted mean is consistently depleted, implying N_2 fixation, and areas in the southwestern tropical region of the basin contain the lowest weighted mean of $\delta^{15}N$ values.*

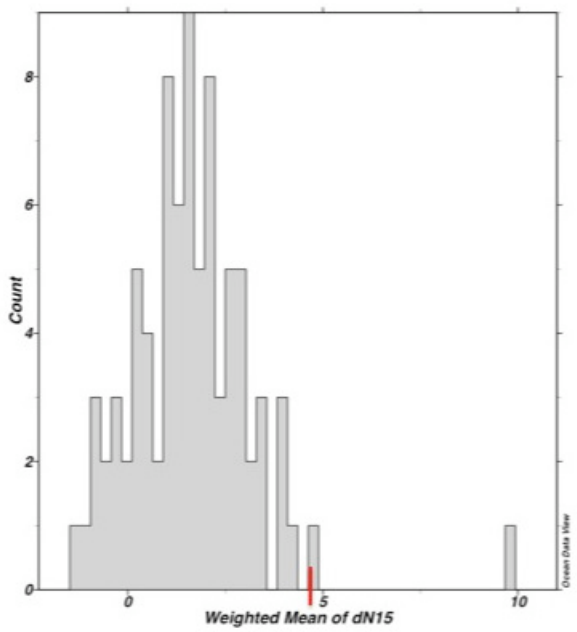

Figure 2B. *Histogram of the weighted means of $\delta^{15}N$ for each station. The $\delta^{15}N$ value of deep-water nitrate (4.5‰) is marked in red. The consistently low $\delta^{15}N$ values show that a N_2 fixation, an isotopically lighter source, is contributing majorly to the N budget in the North Atlantic.*

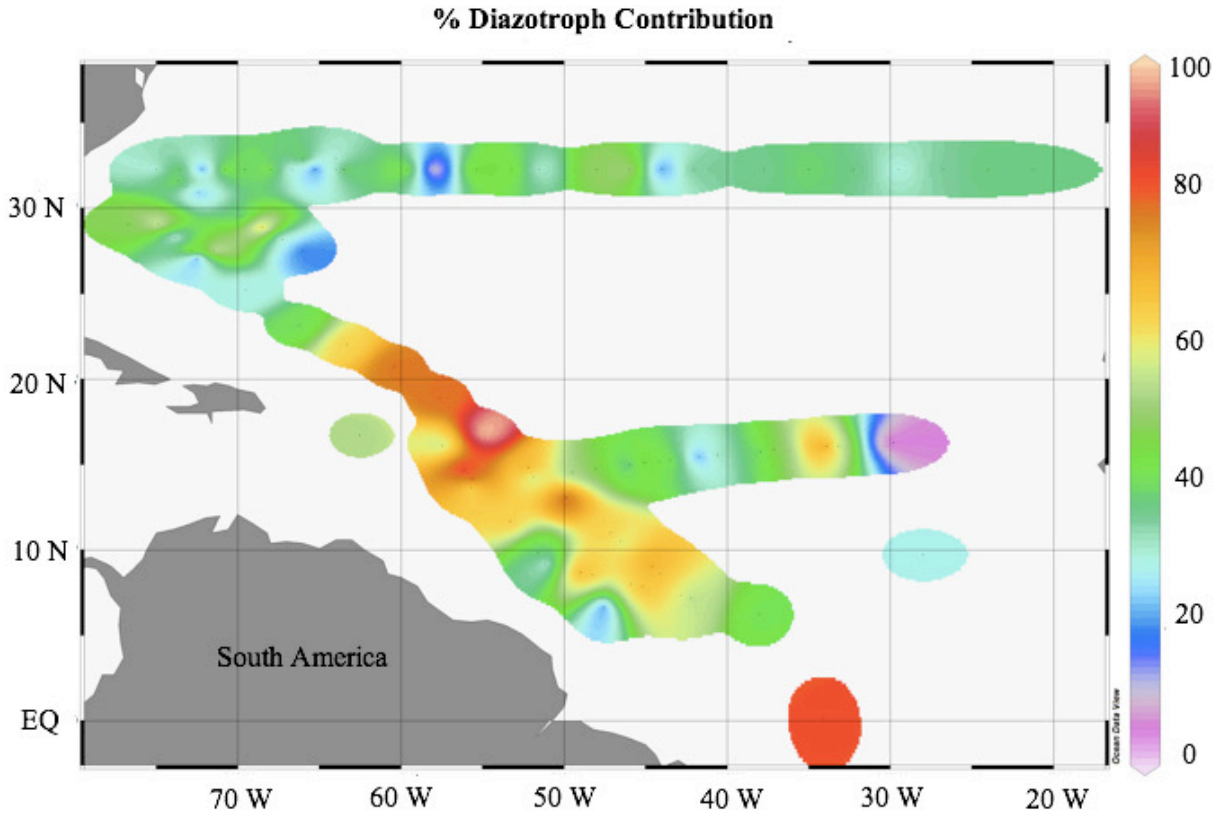

Figure 3. A) *Geographic distribution of the contribution of N_2 fixation in the North Atlantic. The highest contributions from N_2 fixation are in the southwestern regions of the basin, represented by red. Only two stations imply zero contribution from N_2 fixation to the nitrogen budget, station 19 of SJ9603 (29.817 W, 16.283 N) and station 2 of SJ9612 (72.508 W, 27.049 N). White represents area not sampled on the cruises.*

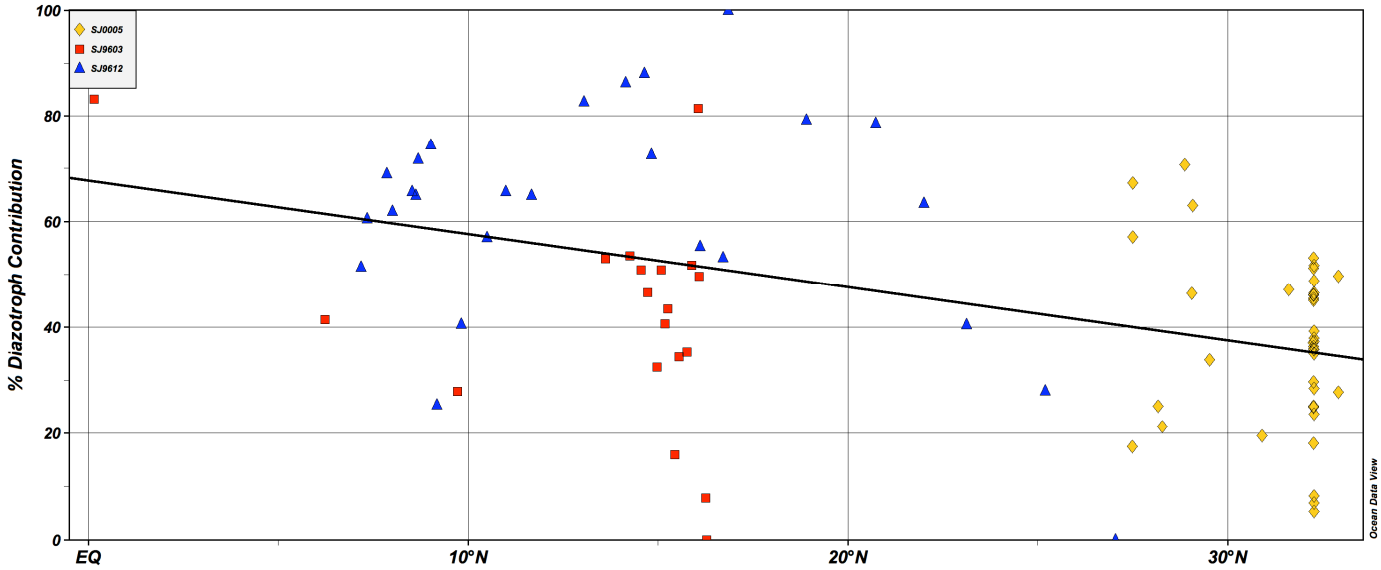

Figure 3. B) *Plot of the contribution of N_2 fixation and latitude of the sample stations. There is a negative correlation between the contribution of N_2 fixation and longitude.*

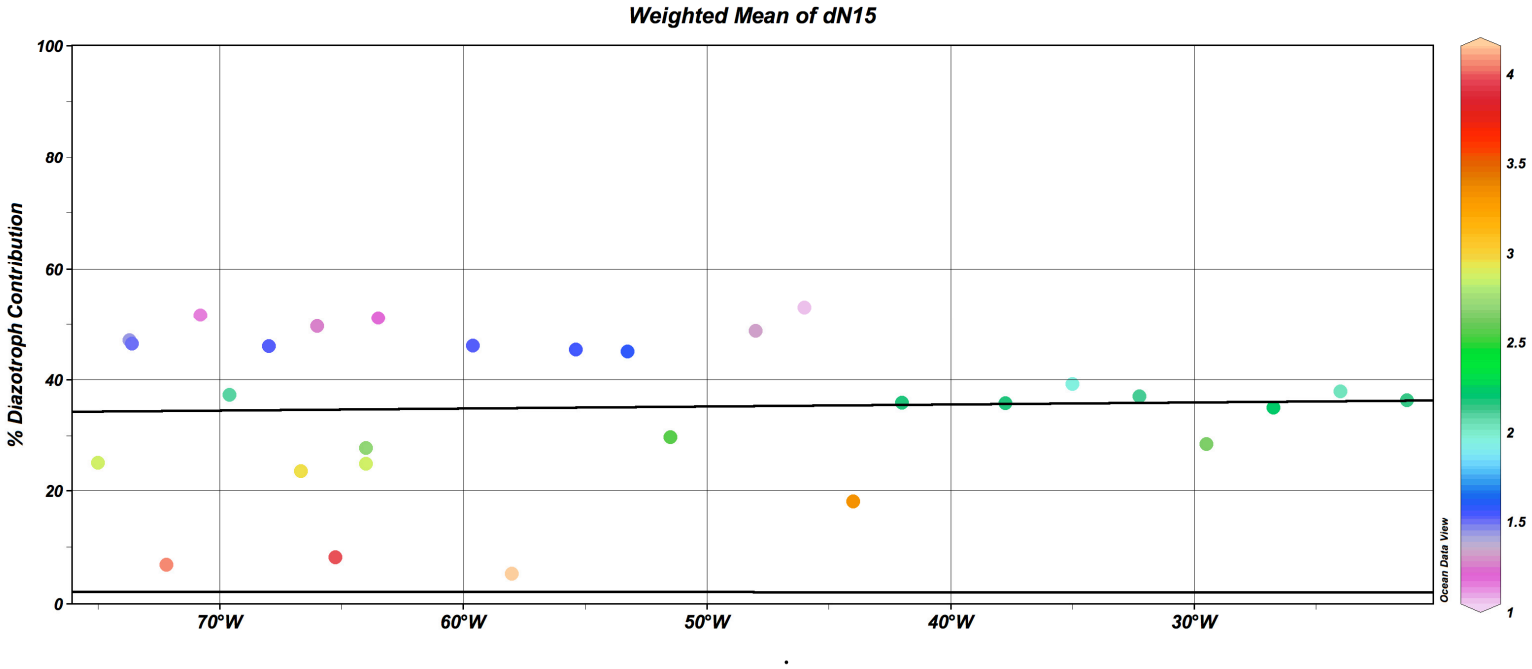

Figure 4. *Contribution of N_2 fixation and longitude.*

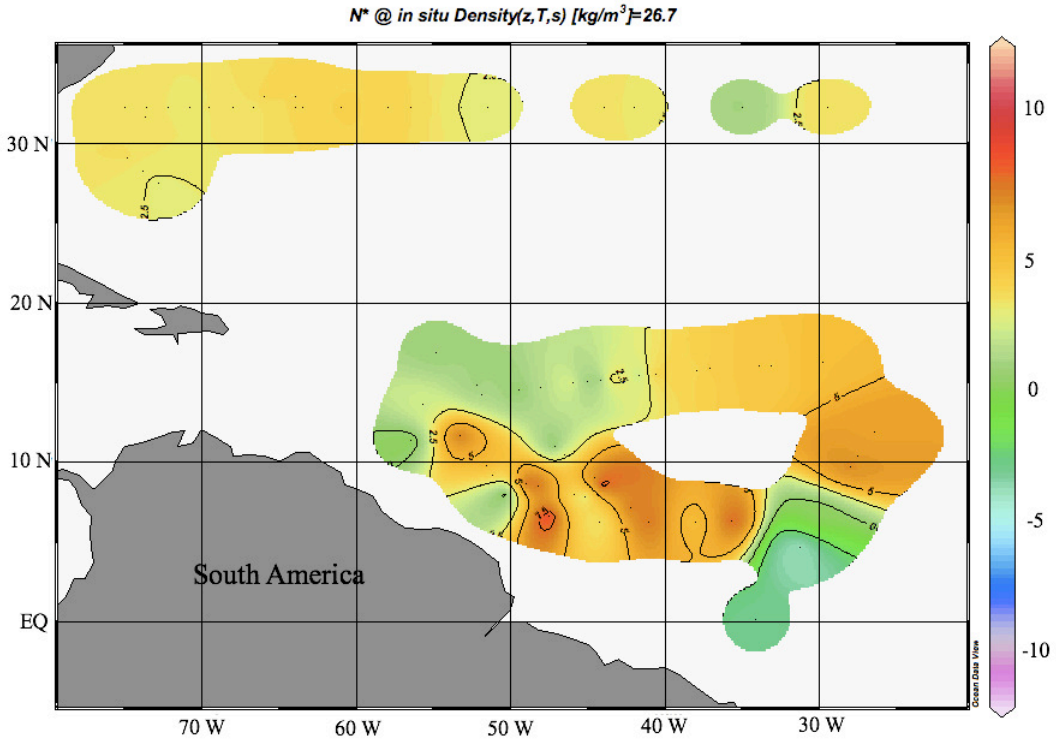

Figure 5: *Geographic distribution of N*. The transbasin transect of SJ0005 at approximately 32 N shows elevated values for N* above 2, with the exception of one station (shown in green). The southwestern region also shows elevated N* vales, depicted in red and orange, with a maximum value of 11.22 mol kg^{-1} located at SJ9612 station 14 (44.086 W, 8.617 N) . High concentrations of N* are also reported for the cruises in the middle of the basin. The persistenly high values of this anomaly strongly implicate N_2 fixation as an important source of new nitrogen to the North Atlantic Ocean.*

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