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8 Large-scale meridional and zonal variability in the nitrogen isotopic composition of plankton in the
9 Atlantic Ocean

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21

22 ABSTRACT

23

24 The zonal (*ca.* 15°-40°W along 26-29°N) and meridional (*ca.* 30°N-30°S along 28°-29°W)
25 variability of $\delta^{15}\text{N}$ of suspended particles and zooplankton ($>40\ \mu\text{m}$) was studied to assess the
26 influence of nitrogen fixation in the isotopic budget of the tropical and subtropical Atlantic ocean.
27 Two cruises were conducted in October-November 2007 and April-May 2008 comprising a zonal
28 and meridional transect each. In the region between 30°-15°N, the concurrently measured nitrogen
29 fixation was insufficient to explain the consistent patch of suspended particles with $\delta^{15}\text{N} < 2\text{‰}$ and
30 points to a significant contribution of atmospheric deposition of light nitrogen to the isotopic
31 budget. The equatorial region (15°N-10°S) is subject to intense nitrogen fixation, which, according
32 to a two-end-member mixing model, may explain 40-60% of the observed $\delta^{15}\text{N}$ in suspended
33 particles and 3-30% in zooplankton. In the South region between 10°S-30°S, low values ($<4\text{‰}$)
34 were measured in suspended particles and zooplankton during 2008. The values of $\delta^{15}\text{N}$ of
35 suspended particles suggest that nitrogen fixation, which is usually low ($<10\ \mu\text{mol N m}^{-2}\ \text{d}^{-1}$), may
36 represent 50-60% of phytoplankton nitrogen in this region. Hence, diazotrophy in the South
37 Atlantic may be more important than previously thought.

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41 INTRODUCTION

42 In many marine ecosystems, primary production is limited by the availability of nitrogen (Vitousek
43 and Howarth, 1991; Karl et al., 1992; Moore et al., 2013). Reactive nitrogen is supplied to the
44 euphotic zone by different physical, chemical and biological processes such as advective diffusion,
45 atmospheric deposition and biological nitrogen fixation. The latter is mediated by organisms and, in
46 the oligotrophic regions of the oceans, is a relevant source of new nitrogen (Paerl and Zehr, 2000).
47 The ratio of stable isotopes in phytoplankton ($^{15}\text{N}:^{14}\text{N}$ expressed as $\delta^{15}\text{N}$ in ‰) is variable, due to
48 the contrasting preferences of the organisms for each isotope. The metabolic pathways usually
49 discriminate against the heavy isotope (^{15}N), a discrimination that is measured by the isotopic
50 fractionation factor (Montoya, 2008). Besides, the different forms of inorganic nitrogen have
51 distinct signatures of $\delta^{15}\text{N}$. Deep-nitrate typically ranges between 3-6‰ (Montoya, 2008),
52 atmospheric dinitrogen is, by definition, 0‰, and deep-ammonium lies between 6-8‰ (Miyake and
53 Wada, 1967). Hence, a very different $\delta^{15}\text{N}$ of organic matter is expected, according to the source of
54 nitrogen, if this is completely consumed. The isotopic signature of phytoplankton will depend then
55 on the signature of the source of nitrogen and the degree of fractionation during uptake. Yet, the
56 interpretation of $\delta^{15}\text{N}$ is not so straightforward. In the case of animals (*i.e.* upper trophic levels), a
57 trophic effect is also observed whereby the tissues of the consumer are usually 2-4‰ heavier than
58 the food, whereas the animal's excreta, mainly in the form of ammonium, can be 2-4‰ lighter than
59 the food (Montoya, 2008; and references herein). In addition, cultured cyanobacteria growing on
60 excess nitrate showed a strong fractionation factor, yielding $\delta^{15}\text{N}$ values similar to those produced
61 by growth on dinitrogen (Bauersachs et al., 2009).

62 In the Atlantic Ocean, experimental data retrieved during large-scale surveys show that
63 *Trichodesmium*, the most well-studied diazotroph, is distributed preferentially between 0-20°N
64 (Tyrrell et al., 2003; Moore et al., 2009; Fernández et al., 2010; Luo et al., 2012). In addition,
65 nitrogen fixation, mostly measured with the method of Montoya et al. (1996), is more significant
66 between 0°-15°N (Moore et al., 2009; Fernández et al., 2010; Luo et al., 2012). The $\delta^{15}\text{N}$ of
67 diazotrophs usually ranges between -1‰ and -2‰ (Montoya et al., 2002). However, the measured
68 isotopic signature of nitrogen in suspended particles and the biogeochemical estimates of excess
69 nitrogen available in the literature suggest that nitrogen fixation is more relevant in a region further
70 north, between 15°-30°N (Gruber and Sarmiento, 1997; Mahaffey et al., 2003; Mahaffey et al.,
71 2004; Reynolds et al., 2007; Hansell et al., 2004). The time scales reflected by these measurements
72 are different: *in situ* nitrogen fixation rates generally represent instantaneous rates over a few hours
73 to 1 day, while $\delta^{15}\text{N}$ and excess nitrogen are indicators of the diazotrophic activity over longer
74 periods of days to months. However, the determinants of this disagreement remain undefined. Duce

75 et al. (2008) argued that the atmospheric deposition of reactive nitrogen in the oceans has increased
76 due to human activities and is fast approaching the marine N₂ fixation budget. Other studies have
77 also shown an increase of the atmospheric deposition of ¹⁵N-depleted nitrogen in high and
78 temperate latitudes (Hastings et al., 2009; Mara et al., 2009; Morin et al., 2009; Holtgrieve et al.,
79 2011), as a result of the increasing anthropogenic production of reactive nitrogen and/or natural
80 speciation processes. In addition, Baker et al. (2007) and Knapp et al. (2010) reported depositional
81 fluxes of low δ¹⁵N similar to measured N₂ fixation rates in the Atlantic Ocean.

82 As part of a wider project, we have previously described the latitudinal and longitudinal distribution
83 of measured community nitrogen fixation in the tropical and subtropical Atlantic Ocean (Fernández
84 et al., 2010; Fernández et al., 2013) and the relative contribution of nitrogen fixation and nitrate
85 eddy diffusion in supplying new nitrogen to the euphotic layer (Mouriño-Carballido et al., 2011).
86 Here we report on the distribution of δ¹⁵N in suspended particles and two size-fractions of plankton,
87 with the aim of describing the large-scale latitudinal variability of nitrogen isotopic signatures in the
88 Atlantic Ocean and comparing these inferred patterns of diazotrophy with concurrent, direct
89 measurements of *in situ* N₂ fixation rates.

90

91 METHODS

92 *Sampling, hydrography and chlorophyll a*

93 Two research cruises were conducted in the tropical and subtropical Atlantic Ocean during 17
94 November-8 December 2007 and 13 April-2 May 2008 on board the BIO ‘Hespérides’. The tracks
95 followed by the vessel comprised a zonal and a meridional transect in each season (Fig. 1).

96 The vertical distribution of temperature, salinity, dissolved oxygen and fluorescence was measured
97 by a SBE 911plus CTD attached to a rosette equipped with 12-L Niskin bottles which was fired to
98 300m depth, always before dawn. The vertical profiles of fluorescence and oxygen at each station
99 were used to choose the sampling depths for the determination of inorganic nutrients concentration,
100 chlorophyll *a* concentration, community ¹⁵N₂ fixation and natural abundance of nitrogen isotopes in
101 suspended particles.

102 The concentration of chlorophyll *a* was measured at 6-7 depths distributed through the euphotic
103 layer. At each depth a 250-mL sample was filtered, using low vacuum pressure, through 0.2 μm
104 pore-size polycarbonate filters. The pigments were extracted overnight in 90% acetone at -4°C.
105 Fluorescence was subsequently measured on board with a Turner Designs 700 fluorometer,
106 calibrated with pure chlorophyll *a* (Fluka).

107

108 Rates of N₂ fixation by the whole planktonic community in a 24-hour incubation period were
109 determined in each station at the surface (5m), an intermediate depth (30-80m) and the depth of the
110 deep chlorophyll maximum (DCM), and are already described in Fernández et al. (2010) and
111 Fernández et al. (2013). Briefly, we incubated triplicate samples following the Montoya et al.
112 (1996) protocol for the ¹⁵N₂-uptake technique with the modifications of Rees et al. (2009). The
113 equations of Weiss (1970) and Montoya et al. (1996) were used to calculate the initial N₂
114 concentration (assuming equilibrium with atmosphere) and N₂ fixation rates, respectively. The limit
115 of detection, estimated following Montoya et al. (1996), was 0.001 μmol N m⁻³ d⁻¹.

116 *Natural abundance of nitrogen isotopes in suspended particles*

117 For the determination of δ¹⁵N signature in suspended particles (δ¹⁵N_{sp}), 2-L samples were taken at 6
118 depths through the euphotic layer in each pre-dawn station and filtered through a 25-mm diameter
119 GF/F filter (Whatman). All filters were dried at 40°C during 24 h and then stored until pelletization
120 in tin capsules. The measurement of particulate organic nitrogen (PON) and ¹⁵N atom% was carried
121 out with an elemental analyzer combined with a continuous-flow stable isotope mass-spectrometer
122 (FlashEA112 + Deltaplus, ThermoFinnigan) and using an acetanilide standard as reference. The
123 limit of detection of the equipment was 0.20 μg N.

124 The isotopic signature observed in the suspended particles may be affected by the presence of other
125 types of material in addition to phytoplankton (*i.e.* bacteria, detritus, zooplankton). The existence of
126 a relationship between the particulate organic nitrogen (PON) to chlorophyll *a* (chl-*a*) ratio and the
127 δ¹⁵N of suspended particles is an indicator of such a trophic effect (Waser et al., 2000). The Pearson
128 product-moment correlation coefficient of PON:chl-*a* and δ¹⁵N_{sp} was calculated to test this
129 possibility.

130 The weighted mean of δ¹⁵N of suspended particles in the euphotic layer was used as an integral of
131 the signature of phytoplankton in the euphotic zone to simplify the comparison with the δ¹⁵N of the
132 two size-fractions of zooplankton (40-200 μm and >200 μm). It was calculated, following Landrum
133 et al. (2011), as:

$$\text{Weighted mean } \delta^{15}\text{N}_{\text{sp}} = \frac{\sum_i([\text{PN}]_i \times \Delta z_i \times \delta^{15}\text{N}_{\text{sp}})}{\sum_i([\text{PN}]_i \times \Delta z_i)}$$

134 Where [PN]_{*i*} is the concentration (μM) of particulate nitrogen, δ¹⁵N_{sp} is the nitrogen isotopic
135 composition of suspended particles (¹⁵N:¹⁴N, ‰), and Δz_{*i*} is the depth interval (m).

136 The fraction contribution of diazotroph nitrogen to the bulk suspended particles defined by
137 Montoya et al. (2002) was also calculated as:

$$\% \text{ diazotroph } N = 100 \times \left(\frac{\delta^{15}\text{N}_{\text{sp}} - \delta^{15}\text{NO}_3^-}{\delta^{15}\text{N}_{\text{diazotroph}} - \delta^{15}\text{NO}_3^-} \right)$$

138 Where $\delta^{15}\text{N}_{\text{diazotroph}}$ is the nitrogen isotopic composition of diazotrophs ($^{15}\text{N}:^{14}\text{N}$, ‰) and $\delta^{15}\text{NO}_3^-$ is
 139 the nitrogen isotopic composition of deep-nitrate ($^{15}\text{N}:^{14}\text{N}$, ‰). As pointed by these authors, this
 140 two-end-member mixing model is sensitive to the values of the end members chosen ($\delta^{15}\text{N}_{\text{diazotroph}}$
 141 and $\delta^{15}\text{NO}_3^-$). In order to represent only the nitrate in the upper thermocline, and avoid the effect of
 142 recently fixed nitrogen recycled between the upper water column and the thermocline in the
 143 calculations, the $\delta^{15}\text{NO}_3^-$ used was 4.5‰, which is the global average of deep-nitrate (Liu and
 144 Kaplan, 1989; Sigman et al., 1997). Due to the fact that most of our stations are oligotrophic, no
 145 additional fractionation factor during nitrate uptake was added. As a conservative choice
 146 representing the least contribution of nitrogen fixers, and considering the fact that little fractionation
 147 occurs during N_2 fixation (Montoya, 2007), the $\delta^{15}\text{N}_{\text{diazotroph}}$ used was -2‰ (Montoya et al., 2002).

148

149 *Natural abundance of nitrogen isotopes in plankton*

150 At each pre-dawn station, zooplankton were collected by vertical tows of a 40 μm net of 30cm in
 151 diameter through the upper 200m of the water column at a constant towing speed of 60 m min^{-1} .
 152 The content of the collector was suspended in 500 mL of 20 μm -filtered seawater. Two 60 mL sub-
 153 samples were preserved, one in Lugol's solution and the other in formaldehyde, for the
 154 determination of abundance of *Trichodesmium* and other plankton by microscopical examination.
 155 *Trichodesmium* trichomes were more abundant in the fraction 40-200 μm while colonies were
 156 present in the >200 μm fraction. The rest of the sample was separated into two size fractions by
 157 passage through nylon sieves of 40 and 200 μm . Each fraction was then re-suspended in 200 mL of
 158 20 μm -filtered seawater and subsequently filtered in pre-weighted 45-mm diameter GF/F filters by
 159 low vacuum pressure. All filters were dried for 24 h at 40°C and stored until measurement of
 160 particulate organic nitrogen and ^{15}N atom% as previously described.

161 The relative contribution of diazotroph N to zooplankton biomass was estimated following Montoya
 162 et al. (2002) two-end-member mixing model for zooplankton:

$$\% \text{ diazotroph } N = 100 \times \left(\frac{\delta^{15}\text{N}_{\text{plankton}} - \delta^{15}\text{N}_{\text{reference pl}}}{\delta^{15}\text{N}_{\text{diazotroph}} - \delta^{15}\text{N}_{\text{reference pl}}} \right)$$

163 Where $\delta^{15}\text{N}_{\text{plankton}}$ stands for the nitrogen isotopic composition of the plankton size-fraction
 164 ($^{15}\text{N}:^{14}\text{N}$, ‰), and $\delta^{15}\text{N}_{\text{reference pl}}$ is the $\delta^{15}\text{N}$ of reference zooplankton. Again, a conservative value of
 165 -2‰ was used for $\delta^{15}\text{N}_{\text{diazotroph}}$. The $\delta^{15}\text{N}$ of the reference plankton was calculated as the mean of
 166 the $\delta^{15}\text{N}_{40}$ or $\delta^{15}\text{N}_{200}$ measured in the stations where the lowest abundance of *Trichodesmium* and

167 nitrogen fixation were found, i.e., the stations between 0-20°S in the latitudinal leg of 2007 cruise,
168 where no influence of nitrogen fixation in the samples is expected. The values used were: 4.6‰ for
169 the $\delta^{15}\text{N}_{\text{reference 40}}$, and 5.9‰ for $\delta^{15}\text{N}_{\text{reference 200}}$. This model is based on the use of reference
170 plankton to account for the trophic effect, i.e., the reference plankton serves as a proxy in both
171 terms of the calculation; therefore, no additional fractionation term for the trophic effect was
172 needed. The assumptions that are implied are: i) the size distribution of grazers in the sample and
173 the reference plankton are similar, ii) the trophic fractionation in the sample and the reference is
174 similar, and iii) in both locations the isotopic composition of the nitrate supporting the food web is
175 the same (J. P. Montoya, Atlanta, personal communication).

176

177 RESULTS

178 *Hydrography and fluorescence*

179 In the latitudinal transects, the Equatorial upwelling was clearly defined by the rising of the isolines
180 of temperature (T) and salinity (S) in both cruises (Fig. 2a, b, c, d). In order to simplify the analysis
181 of data, we use the changes in the depth of the 16°C isotherm, above and below 150m, to identify
182 the area affected by the Equatorial upwelling and delimit three main regions along the transects,
183 i.e., North gyre (29°-15°N), equatorial region (15°N-10°S) and South gyre (10°-33°S).

184 The hydrographic settings found in these regions were similar in both legs. Surface waters in the
185 equatorial region were always warmer (> 24°C) and less saline (< 35 psu) than in the gyres in both
186 seasons. In turn, the stability of the water column in the gyres was weaker than that found in the
187 equatorial region, where the average Brunt-Väisälä frequency in the upper 125m was higher
188 (Fernández et al., 2010). The fluorescence profiles showed a well-defined deep chlorophyll
189 maximum (DCM) associated with the thermocline in both transects (Fig. 2e, f). This DCM was
190 shallower and better defined in the equatorial region than in the gyres. By contrast, in the
191 longitudinal sections, waters were warmer and slightly more saline in autumn 2007 than in spring
192 2008 (Fig. 2e), leading to a stronger stability of the water column, as indicated by the higher Brunt-
193 Väisälä frequency measured in this cruise (Fernández et al., 2013). The DCM was located at *ca.*
194 100m in both zonal legs and no apparent trend in depth was observed (Fig. 2e, f).

195

196 *Stable nitrogen isotopes in suspended particles ($\delta^{15}\text{N}_{\text{sp}}$) and particulate organic nitrogen (PON)*

197 The Pearson product-moment correlation coefficient of PON:chl-*a* and $\delta^{15}\text{N}_{\text{sp}}$ showed no significant
198 relationship, neither positive nor negative, in our two cruises (Fig. 3). The PON:chl-*a* ratio
199 represents the contribution of other components of the food web than phytoplankton. If any

200 relationship between PON:chl-*a* ratio and $\delta^{15}\text{N}_{\text{sp}}$ is found, this will suggest a significant effect of
201 detrital matter and/or other non-phytoplanktonic organisms on the observed signal of suspended
202 particles. Thus, we can assume that the $\delta^{15}\text{N}$ of suspended particles in our data mainly reflects the
203 composition of phytoplankton (Waser et al., 2000).

204 The zonal distribution of PON showed no apparent trend and the measured concentrations were
205 similar in magnitude in both cruises (Fig. 4b, d), without any significant differences between cruises
206 (t-test, $n=60$). The mean PON concentrations for the zonal transects were $0.22\pm 0.12\ \mu\text{M}$ in 2007
207 and $0.24\pm 0.06\ \mu\text{M}$ in 2008. In the latitudinal sections, the measured concentrations in the South
208 gyre were similar in both cruises, showing similar values and vertical variability (Fig. 4a, c). In the
209 North gyre and equatorial regions, measured PON differed significantly between cruises (t-test, $p <$
210 0.01 , $n=54$ and $p < 0.05$, $n=84$, respectively). In the North gyre region, the concentrations of PON
211 in 2007 cruise were higher than that of 2008 cruise. In the equatorial region, PON depicted a
212 decreasing pattern in 2007 cruise, but no linear trend was observed in 2008.

213 In the zonal transect of 2007 cruise, the $\delta^{15}\text{N}_{\text{sp}}$ increased sharply by 4-6‰ from 80-100m to the base
214 of the euphotic layer, probably reflecting the influence of the African upwelling in the easternmost
215 stations (Fig. 5a). However, the $\delta^{15}\text{N}_{\text{sp}}$ distributed uniformly in the euphotic layer in 2008 cruise
216 (Fig. 5b). In the latitudinal transects, the $\delta^{15}\text{N}_{\text{sp}}$ was lower in 2007 than in 2008, both in the North
217 gyre (Fig 5c, d) and equatorial region (Fig 5e, f). By contrast, in the South gyre region, the vertical
218 distribution of $\delta^{15}\text{N}_{\text{sp}}$ differed between stations in each cruises (Fig 5g, h), with a wide range of
219 values between -4 and 4% . The difference between cruises were significant in the zonal transect,
220 the North gyre and the equatorial region (t-test, $p < 0.01$, $n=72$), but not in the South gyre region.

221 The correlations of $\delta^{15}\text{N}$ of suspended particles with ammonium concentration and with nitrate
222 concentration are shown in Table 1. Considering all the stations in each cruise, $\delta^{15}\text{N}_{\text{sp}}$ correlated
223 with ammonium concentration in 2007 ($p < 0.05$, $n=128$) and with nitrate in 2008 ($p < 0.05$,
224 $n=119$).

225

226 *Nitrogen isotopic signature in the euphotic layer*

227 In order to compare the $\delta^{15}\text{N}$ of suspended particles and plankton net tows, we calculated the
228 weighted mean of $\delta^{15}\text{N}$ of suspended particles to obtain an integrative $\delta^{15}\text{N}_{\text{sp}}$ signature for the whole
229 euphotic layer. The patterns described by the $\delta^{15}\text{N}$ of the planktonic 40-200 μm ($\delta^{15}\text{N}_{40}$) and >200
230 μm ($\delta^{15}\text{N}_{200}$) size-fractions were very similar in all stations, with a few exceptions in the equatorial
231 region and the zonal legs, and closely matched that of $\delta^{15}\text{N}_{\text{sp}}$ (Fig. 6). The average differences
232 between $\delta^{15}\text{N}_{\text{sp}}$ and the two plankton size fractions were in the range previously described

233 (Minagawa and Wada, 1984): between the $\delta^{15}\text{N}_{\text{sp}}$ and the $\delta^{15}\text{N}_{40}$ that difference was 3.2‰ in 2007
234 and 2.6‰ in 2008; between the $\delta^{15}\text{N}_{\text{sp}}$ and the $\delta^{15}\text{N}_{200}$ was 4.3‰ in 2007 and 1.9‰ in 2008.

235 In the autumn 2007 meridional transect, the isotopic signature of suspended particles showed two
236 minima ($< -2\text{‰}$) in the North gyre and South gyre regions. In the equatorial region, $\delta^{15}\text{N}_{\text{sp}}$
237 oscillated around 0‰ (Fig. 6). The $\delta^{15}\text{N}_{40}$ and $\delta^{15}\text{N}_{200}$ roughly followed these patterns. By contrast,
238 the distributions were dome-shaped in spring 2008, reaching peak values in the equatorial region. In
239 both cruises, the gyres presented low $\delta^{15}\text{N}$ values in most of the stations. Besides, a positive
240 statistical correlation between $\delta^{15}\text{N}$ in the three fractions suggests a regular impact of light nitrogen
241 across trophic levels (Table 2).

242 A two-way factorial ANOVA indicated significant differences between regions and cruises, and for
243 $\delta^{15}\text{N}_{40}$ and $\delta^{15}\text{N}_{200}$, a significant interaction region-cruise, which enhances the difference (Table 3).
244 The differences between regions appeared to be significant only for the North gyre-equatorial
245 region (post-hoc Tukey HSD test), as can be also seen in figure 6.

246 We tried to estimate if *Trichodesmium* could be the major influence on the patterns observed but no
247 significant correlation (Pearson's r) was found between the measured filament abundance
248 (Fernández et al., 2010; Fernández et al., 2013) and the $\delta^{15}\text{N}$ of suspended particles ($p=\text{n.s.}$, $n=42$),
249 the 40-200 μm ($p=\text{n.s.}$, $n=41$) or the >200 μm plankton size-fractions ($p=\text{n.s.}$, $n=42$).

250 Nitrogen fixation rates (Fig. 6) were previously reported in Fernández et al. (2010) and Fernández
251 et al. (2013). Briefly, in the longitudinal transects no apparent trend was depicted in 2007, while a
252 clear increasing pattern to the East appeared in 2008 (Fig. 6b, d). In the spring 2008 zonal leg, the
253 average vertically integrated N_2 fixation was 7-fold higher than that of autumn 2007 (8.3 ± 3.3 μmol
254 $\text{N m}^{-2} \text{d}^{-1}$ vs. 1.2 ± 0.5 $\mu\text{mol N m}^{-2} \text{d}^{-1}$). In both meridional transects, the highest integrated rates (*ca.*
255 250 and 150 $\mu\text{mol N m}^{-2} \text{d}^{-1}$ in 2007 and 2008, respectively) were measured at stations located
256 within the equatorial region (Fig. 6a, c). Besides, the North gyre showed higher diazotrophic
257 activities than the South gyre. But, while N_2 fixation south of the Equator was almost undetectable
258 during the 2007 cruise, substantial rates were measured in the Southern Hemisphere in 2008 (Fig.
259 6a, c).

260

261 *Diazotroph nitrogen contribution to $\delta^{15}\text{N}$ in the euphotic layer*

262 The contribution of diazotrophs to the observed $\delta^{15}\text{N}$ of suspended particles, 40-200 μm and >200
263 μm plankton size-fractions, estimated by the two-end-member mixing models, decreased to the
264 South in 2007 cruise (Table 4). In 2008, the minimum was observed in the equatorial region (Table
265 4). The importance of this contribution is higher in 2007, except in the South gyre, where the

266 contribution of diazotroph nitrogen was higher in all size-fractions. In both cruises, diazotrophy
267 explains, on average, 61% of the observed $\delta^{15}\text{N}_{\text{sp}}$; 27% of $\delta^{15}\text{N}_{40}$, and 30% of $\delta^{15}\text{N}_{200}$.

268

269 DISCUSSION

270 Our data contributes to the existing studies in the Atlantic Ocean (Waser et al., 2000; Mino et al.,
271 2002; Montoya et al., 2002; Mahaffey et al., 2003; Mahaffey et al., 2004; Reynolds et al., 2007;
272 Landrum et al., 2011; Mompeán et al., 2013) providing basin-scale distribution of $\delta^{15}\text{N}$ in
273 suspended particles and two plankton size-fractions during two contrasting seasons. We found a
274 consistent ^{15}N -depleted signal ($<4\text{‰}$) in suspended particles ($\delta^{15}\text{N}_{\text{sp}}$) in the euphotic layer in most
275 of the stations (Fig. 5; Fig. 6). This implies that nitrogen fixation and/or atmospheric deposition
276 were supplying an important fraction of new nitrogen in most of the tropical and subtropical
277 Atlantic Ocean in our cruises. The trends in the $\delta^{15}\text{N}$ of the two plankton size-fractions closely
278 matched that of suspended particles, indicating a sensible impact of light nitrogen even in upper
279 trophic levels, at least for some regions (Fig. 6, Table 2).

280 In the meridional transect of 2008, the $\delta^{15}\text{N}$ signal in the $>200\mu\text{m}$ size-fraction ($\delta^{15}\text{N}_{200}$) was lower
281 than that in the $40\text{-}200\mu\text{m}$ size-fraction ($\delta^{15}\text{N}_{40}$) in most of the stations (Fig. 6), contrary to the
282 usual observed enrichment in ^{15}N of upper trophic levels (Montoya, 2008). One possible reason is
283 the longer turnover times of mesozooplankton relative to phytoplankton and microplankton that
284 could result in the uncoupling of different size-fractions, producing this inversion of the expected
285 increasing pattern (Landrum et al., 2011; Mompeán et al., 2013). Other possible explanation could
286 be the presence of *Trichodesmium* colonies, which were large enough to be retained in this size
287 fraction, and would have lowered the isotopic signature of zooplankton.

288

289 *Zonal and meridional variations in $\delta^{15}\text{N}$ in the North gyre*

290 In the North gyre, the difference in $\delta^{15}\text{N}_{\text{sp}}$ between stations was higher in autumn 2007 than in
291 spring 2008 (6‰ and 2‰, respectively). However, the vertical change in $\delta^{15}\text{N}_{\text{sp}}$ at each station was
292 small in both cruises (Fig. 5), and the measured values of $\delta^{15}\text{N}_{\text{sp}}$ were always $<2\text{‰}$. Our data are in
293 agreement with previous reports in the subtropical North Atlantic which show a range of variation
294 between -2 and 4‰ in the signature of suspended particles (Montoya et al., 2002; Mahaffey et al.,
295 2003; Reynolds et al., 2007; Landrum et al., 2011). These authors described a consistently depleted
296 signal between 7° and 32°N , which is also confirmed by geochemical tracers (Gruber and
297 Sarmiento, 1997; Hansell et al., 2004) that point to a persistent excess nitrate relative to phosphate
298 in this area, indicative of intense nitrogen fixation. These light nitrogen signatures have been

299 associated with a large impact of diazotrophic nitrogen in the isotopic budget of this area,
300 discarding the influence of other sources of new light nitrogen because of their weak strength or
301 unlikely occurrence (Reynolds et al., 2007; Landrum et al., 2011). However, later studies provided
302 new insights that suggest a more relevant influence of alternative sources such as the atmospheric
303 deposition of ^{15}N -depleted nitrogen (Baker et al., 2007; Hastings et al., 2009; Morin et al., 2009;
304 Mara et al., 2009; Knapp et al., 2010; Holtgrieve et al., 2011; Mouriño-Carballido et al., 2011)

305 The $\delta^{15}\text{N}$ of deep-water nitrate typically ranges between 3-6‰ with a global average of 4.8‰
306 (Montoya, 2008). In the presence of excess nitrate, the isotopic fractionation, due to the incomplete
307 exhaustion of the nitrate pool by phytoplankton, could result in values of $\delta^{15}\text{N}_{\text{sp}}$ lower than 3‰
308 (Montoya, 2008). A recent study showed that cyanobacteria, especially *Trichodesmium*, growing on
309 nitrate could express a nitrogen isotopic signal similar to that of nitrogen fixation depending on the
310 isotopic composition of the nitrogen source, the degree of fractionation, and the species of
311 cyanobacterium (Bauersachs et al., 2009). However, no excess dissolved inorganic nitrogen was
312 found in surface waters in our zonal or meridional legs, where the concentration of nitrate in the
313 euphotic layer was always lower than 130 nM (Mouriño-Carballido et al., 2011; Fernández et al.,
314 2010; Fernández et al., 2013). We also recorded measurable but low abundances of *Trichodesmium*
315 (<60 trichomes L^{-1}) in the euphotic layer (Fernández et al., 2010; Fernández et al., 2013), which is
316 also an indication of potential diazotrophy in the area. But, no significant correlation appeared
317 between *Trichodesmium* abundances and the $\delta^{15}\text{N}_{\text{sp}}$ (Pearson's r). Hence, we would not expect that
318 a strong isotopic fractionation associated with cyanobacteria or other phytoplankters was
319 responsible for the observed $\delta^{15}\text{N}_{\text{sp}}$ during our cruises.

320 The lack of data on atmospheric deposition of nitrogen during our study limits any direct
321 comparison with the measured nitrogen fixation and the distribution of the $\delta^{15}\text{N}$ signature, but we
322 can attempt to use an indirect analysis instead. The two-end-member model proposed by Montoya
323 et al. (2002) yields a contribution of N_2 fixation to $\delta^{15}\text{N}_{\text{sp}}$ in the range 81-85% in 2007 and 59-61%
324 in 2008 (Table 4), which is close to the previous estimation of 74% by Reynolds et al. (2007).

325 However, experimental measurements of community nitrogen fixation in this region indicate
326 modest rates of diazotrophy ($<60 \mu\text{mol N m}^{-2} \text{d}^{-1}$) throughout the year (Fig. 6; Moore et al., 2009;
327 Benavides et al., 2011). During the spring 2008 cruise, Mouriño-Carballido et al. (2011) calculated
328 the relative importance of nitrate eddy diffusion and measured rates of nitrogen fixation as sources
329 of new nitrogen to the euphotic layer in the North gyre. They estimated that the average
330 contribution of nitrogen fixation in this cruise was only 2% over daily time-scales. We acknowledge
331 that the comparison of these two fluxes is difficult as they represent different time-scales, *i.e.*, the
332 $\delta^{15}\text{N}_{\text{sp}}$ represents time-scales of days to weeks, while the measured nitrogen fixation time-scale is

333 one day. But the difference between the fluxes was 30-fold in 2008. This suggests that other sources
334 than diazotrophy may be contributing to our observed $\delta^{15}\text{N}_{\text{sp}}$, and that the low values are not only a
335 consequence of intense nitrogen fixation. The importance of the atmospheric deposition of low $\delta^{15}\text{N}$
336 nitrogen, natural or anthropogenic, is increasing in high and temperate latitudes (Hastings et al.,
337 2009; Morin et al., 2009; Mara et al., 2009; Holtgrieve et al., 2011). According to the model of
338 Duce et al. (2008), the atmospheric supply of anthropogenic reactive nitrogen in the central North
339 Atlantic is usually higher in the latitudinal range between 5° and 25°N . The $\delta^{15}\text{N}$ of this
340 anthropogenic N depends on its origin and is extremely variable (Fang et al., 2011). For instance,
341 the $\delta^{15}\text{N}$ of fuel NO_x produced by power plants ranges between 5 and 13‰ (Heaton, 1990; Kiga et
342 al., 2000), but that of thermal NO_x produced by vehicle exhausts ranges between -13 and -2 ‰
343 (Heaton, 1990). Besides, in the Atlantic Ocean between 45°N - 45°S , Morin et al. (2009) found a
344 $\delta^{15}\text{N}$ of atmospheric nitrate that ranged between -7 and -1.6 ‰ and was mainly representing natural
345 sources. In the Mediterranean Sea (Crete Island), Mara et al. (2009) described a consistent source of
346 low $\delta^{15}\text{N}$ nitrate throughout the year with a potential impact on the isotopic budget of intermediate
347 and deep waters, which could lead to an overestimation of N_2 fixation if atmospheric nitrate is
348 neglected. In spite of that, previous studies discarded the effect of this process in the analysis of the
349 nitrogen isotopic budget, based on the assumption that the flux is small compared to nitrogen
350 fixation (Landrum et al., 2011) or to the export flux of nitrogen out of the euphotic layer (Reynolds
351 et al., 2007). By contrast, Baker et al. (2007) and Knapp et al. (2010) measured atmospheric
352 depositional fluxes of ^{15}N -depleted nitrogen similar to those of N_2 fixation in the North Atlantic
353 Ocean. Considering this information, we suggest that the observed $\delta^{15}\text{N}_{\text{sp}}$ in the North gyre region
354 during our cruises could be the result of the supply of light nitrogen through both nitrogen fixation
355 and atmospheric deposition. Therefore, discarding the effect of this atmospheric supply in the
356 analysis of $\delta^{15}\text{N}_{\text{sp}}$ would result in the overestimation of nitrogen fixation.

357 The signature of light nitrogen found in suspended spread over the food web. Firstly, the $\delta^{15}\text{N}$
358 distributions of the two zooplankton size-fractions and the suspended particles are significantly
359 correlated (Fig. 6, Table 2). Secondly, the observed difference between plankton and particles at
360 each station (2-4‰) is in agreement with previous studies reporting that zooplankton nitrogen is
361 typically 3‰ heavier than phytoplankton (Minagawa and Wada, 1984) due to the enrichment of
362 zooplankton tissues by the isotopic fractionation associated with metabolic and excretory processes
363 (Montoya, 2008). Thirdly, both plankton size-fractions showed relatively low values of $\delta^{15}\text{N}$
364 throughout the transects (<4 ‰). Again, the data provided by the two-end-member model proposed
365 by Montoya et al. (2002) point out that the contribution of nitrogen fixation to upper trophic levels
366 in 2007 represents 48-52% in the 40-200 μm size-fraction and 41-43% in the >200 μm size-fraction
367 (Table 4). In 2008, it represented roughly 16-21% of the 40-200 μm fraction signal and the 31-36%

368 of the >200 μm size-fraction (Table 4). However, we would expect an overestimation of this
369 contribution due to the combined effect of atmospheric deposition of ^{15}N -depleted nitrogen and
370 nitrogen fixation in the isotopic budget of this region.

371 The $\delta^{15}\text{N}$ measured in suspended particles and zooplankton suggests a consistent supply of light
372 nitrogen in this region of the Atlantic Ocean throughout the year, which coincides with previous
373 studies (Montoya et al., 2002; Mahaffey et al., 2003; Reynolds et al., 2007; Landrum et al., 2011).
374 However, we did not measure the isotopic composition of the depositional fluxes, which could be
375 compared with measured community nitrogen fixation in our cruises to determine the actual
376 contribution of each flux (Baker et al., 2007; Knapp et al., 2010). Further studies, characterizing the
377 strength, frequency and $\delta^{15}\text{N}$ of the atmospheric sources of nitrogen relative to *in situ* measured
378 nitrogen fixation, will help to unequivocally ascertain the relative importance of each process in
379 determining the $\delta^{15}\text{N}$ signatures in the North Atlantic.

380

381 *Meridional variations in $\delta^{15}\text{N}$ in the equatorial region*

382 In the equatorial region (15°N-10°S), the meridional trends of the $\delta^{15}\text{N}$ of suspended particles
383 coincided with those previously described by Mahaffey et al. (2004). However, our absolute values
384 are lower than theirs, in the range -2 to 4‰ , and closer to those measured by Reynolds et al. (2007)
385 in the water column and by Mino et al. (2002) in surface waters. The upwelling in this region allows
386 a persistent diffusion of deep nitrate to surface waters, which was reflected in the increase of nitrate
387 concentration during our cruises (Fernández et al., 2010; Mouriño-Carballido et al., 2011;
388 Fernández et al., 2013), and is likely to support a substantial fraction of primary production.
389 Therefore, heavy deep-nitrate is probably determining part of the $\delta^{15}\text{N}$ of suspended particles in the
390 equatorial region.

391 In autumn 2007, the difference between the $\delta^{15}\text{N}$ of 40-200 μm and >200 μm plankton size-
392 fractions (2‰) suggests either a different time scale in the integration of the signal or a low
393 efficiency in the transference of nitrogen to upper trophic levels. The latter could be attributed to the
394 loss of isotopically light ammonium through excretory processes, which was suggested as a major
395 source of light nitrogen in oligotrophic regions (Checkley and Miller, 1989; Montoya, 2008).
396 However, the positive correlation between $\delta^{15}\text{N}$ of suspended particles and ammonium
397 concentration in our cruises (Table 1) suggests that the increase in ammonium is increasing the
398 $\delta^{15}\text{N}_{\text{sp}}$ and may not be related to the excretion of plankton.

399 The cyanobacterium *Trichodesmium* exudates up to 50% of recent fixed N_2 as dissolved organic
400 nitrogen, which can be easily assimilated by other phytoplankters and/or bacteria (Glibert and

401 Bronk, 1994). Furthermore, both nitrogen fixation (Fig. 6) and *Trichodesmium* abundances typically
402 reach high values in this region (Tyrrell et al., 2003; Moore et al., 2009; Fernández et al., 2010).
403 Thus, the supply of light ammonium linked to diazotrophs is probably determining an important
404 fraction of the nitrogen isotopic budget in the equatorial region. The two-end-member mixing
405 model (Montoya et al., 2002) yields an average contribution of this diazotroph nitrogen to $\delta^{15}\text{N}_{\text{sp}}$ of
406 $62\pm 27\%$ in autumn 2007 and $39\pm 8\%$ in spring 2008 (Table 4). On the other hand, Mouriño-
407 Carballido et al. (2011) estimated that the daily contribution of N_2 fixation to total (N_2 fixation +
408 vertical diffusion of nitrate) input of new nitrogen was 22% in the 2008 cruise. Again, these fluxes
409 represent different time scales, but both suggest that nitrogen fixation account for a relevant fraction
410 of the supply of nitrogen to the euphotic layer in this region, and are consistent with previous
411 experimental measurements.

412 The diazotroph nitrogen was inefficiently transferred to upper trophic levels, as it accounted for
413 $25\pm 18\%$ in the 40-200 μm size-fraction and $11\pm 3\%$ in the $>200 \mu\text{m}$ size-fraction in 2007 cruise,
414 and for $3\pm 2\%$ in the 40-200 μm size-fraction and $29\pm 12\%$ in the $>200 \mu\text{m}$ size-fraction in 2008
415 cruise (Table 4). *Trichodesmium*, the dominant diazotroph in this region, is toxic to many species of
416 zooplankton (Hawser et al., 1992) and only a few groups of copepods are known to graze it (O'Neil
417 and Roman, 1994). Besides, these groups seem to excrete a major fraction of the ingested nitrogen
418 (O'Neil et al., 1996, Wannicke et al., 2010). Thus, diazotroph nitrogen is preferentially transferred
419 through dissolved pools when *Trichodesmium* dominates the community (Mulholland, 2007).

420

421 *Meridional variations in $\delta^{15}\text{N}$ in the South gyre region*

422 The distribution of $\delta^{15}\text{N}$ of suspended particles ($\delta^{15}\text{N}_{\text{sp}}$) and zooplankton in the South gyre region
423 depicted contrasting trends in 2007 and 2008 (Fig. 6). Even though the vertical distribution of
424 $\delta^{15}\text{N}_{\text{sp}}$ varied within a range of 6‰, data were $<4\%$ in all stations (Fig. 5) suggesting that a ^{15}N -
425 depleted source of nitrogen is significantly contributing to the signals. In autumn 2007, the general
426 meridional pattern largely coincided with that described by Mino et al. (2002) in surface waters, but
427 not with those given by Mahaffey et al. (2004) and Reynolds et al. (2007), who found a general
428 increasing trend to the South with values $>2\%$. The light patch of $\delta^{15}\text{N}_{\text{sp}}$ in the range -2 to 0% ,
429 which was found between 20° - 30°S in the 2007 cruise (Fig. 6), seems to be a persistent feature also
430 described by Mino et al. (2002) with values close to -1% , and Reynolds et al. (2007) with values
431 close to 0% . By contrast, this is the first time that a decreasing trend in $\delta^{15}\text{N}$ such as the one
432 depicted in spring 2008 is described in this region.

433 The flux of atmospheric deposition of nutrients in the South Atlantic Ocean is extremely weak (Gao
434 et al., 2001; Duce et al., 2008), thus we may discard the effect of light atmospheric nitrogen in the

435 isotopic budget. The small difference in the $\delta^{15}\text{N}$ of both zooplankton size-fractions suggests a high
436 coupling between trophic levels, with low isotopic fractionation in the loss of nitrogen by excretion
437 (Checkley and Miller, 1989). The uptake of dissolved organic nitrogen and their inorganic
438 degradation products, originated by the nitrogen fixers and processed by microbes, may explain
439 such coupling, as isotopic fractionation in microbial food webs is generally low (Rau et al., 1990).
440 Mahaffey et al. (2004) suggested that the relatively important dissolved organic nitrogen pool of the
441 South Atlantic could account for the high $\delta^{15}\text{N}$ measured in their study. On the contrary, Knapp et
442 al. (2011) found that a long-lived and poorly reactive DON pool in other regions of the Atlantic and
443 Pacific Oceans which could be a source of light ammonium through deamination. However, we
444 propose that the supply of diazotroph nitrogen is significantly determining the observed $\delta^{15}\text{N}_{\text{sp}}$. The
445 few experimental measurements performed to date in the South Atlantic show that nitrogen fixation
446 is persistent in this region with rates in the range 2 to 50 $\mu\text{mol N m}^{-2} \text{d}^{-1}$ (Moore et al., 2009;
447 Fernández et al., 2010; Grosskopf et al., 2012), which are similar to those reported in the equatorial
448 and North gyre region (Fig. 6). This suggests that nitrogen fixation could be responsible of the
449 persistent light patch of $\delta^{15}\text{N}_{\text{sp}}$ in the South gyre between 10°-30°S. The contribution of nitrogen
450 fixation to the $\delta^{15}\text{N}$ of suspended particles was $49\pm 12\%$ in 2007 and $58\pm 18\%$ in 2008, according to
451 a two-end-member model based on nitrate and diazotrophy (Montoya et al., 2002). Besides, the
452 daily contribution of nitrogen fixation to the total (N_2 fixation + nitrate diffusive flux) input of
453 nitrogen to the euphotic layer was 44% during strong stratification conditions in April 2008
454 (Mouriño-Carballido et al., 2011).

455 This diazotroph nitrogen is transferred to upper trophic levels with relatively high efficiency in
456 2008 and it represented $36\pm 16\%$ of the 40-200 μm size-fraction and $40\pm 17\%$ of the >200 μm size-
457 fraction (Table 4). Hence, both experimental measurements and estimations seem to agree in that
458 nitrogen fixation could be supporting an important fraction of primary production in the South gyre,
459 despite the fact that the absolute rates of both processes are low.

460

461 *Conclusions*

462 A persistent and consistent signature of low $\delta^{15}\text{N}$ of suspended particles ($\delta^{15}\text{N}_{\text{sp}}$) is found in the
463 North gyre region (30°-15°N) in both zonal and meridional transects, which is usually associated
464 with a relevant input of nitrogen fixed by diazotrophs (Mahaffey et al., 2003; Reynolds et al., 2007;
465 Landrum et al., 2011). However, the experimental measurements of nitrogen fixation show modest
466 rates in comparison with other regions of the Atlantic Ocean and do not seem to support this
467 argument. The atmospheric deposition of light nitrogen, which is increasing in the last years, is
468 likely to complete the required supply that produces this depleted $\delta^{15}\text{N}$ signal. However, few studies

469 have addressed the depositional and diazotrophic fluxes together (Baker et al., 2007; Knapp et al.,
470 2011) and further studies are needed to accurately define the strength, frequency and isotopic
471 composition of the atmospheric depositional flux against the flux of nitrogen fixation in the North
472 Atlantic. The equatorial region (15°N-10°S) is subject to relatively intense nitrogen fixation
473 throughout the year (Moore et al., 2009; Fernández et al., 2010; Grosskopf et al., 2012) which may
474 explain 40 to 60% of the observed $\delta^{15}\text{N}_{\text{sp}}$ signal. However, this nitrogen of diazotrophic origin
475 seems to be inefficiently transferred to upper trophic levels. In the South gyre, the low $\delta^{15}\text{N}_{\text{sp}}$ and
476 the daily estimated contribution of nitrogen fixation to the supply of new nitrogen (Mouriño-
477 Carballido et al., 2011) suggest that diazotrophs can contribute up to half of the nitrogen in
478 phytoplankton at different time scales (Fig. 4). Even though the measured nitrogen fixation rates are
479 low (Moore et al., 2009; Fernández et al., 2010; Grosskopf et al., 2012), their impact in the nitrogen
480 isotopic budget of this region may be large. Hence, a re-evaluation of the importance of diazotrophy
481 in the South Atlantic Ocean is needed through new studies that should address the annual variability
482 in nitrogen fixation rates as well as the distribution and relative importance of the different groups
483 of diazotrophs.

484

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630

631 Table and figure legends

632 Table 1. Pearson product-moment correlation coefficient between $\delta^{15}\text{N}$ of suspended particles
633 ($\delta^{15}\text{N}_{\text{sp}}$) and nutrient concentration: ammonium (NH_4) and nitrate (NO_3), in 2007 and 2008 cruises.
634 Numbers in brackets represent the total number of samples used for the analysis.

635 Table 2. Pearson product-moment correlation coefficient between $\delta^{15}\text{N}$ of suspended particles
636 ($\delta^{15}\text{N}_{\text{sp}}$), $\delta^{15}\text{N}$ of 40-200 μm plankton size-fraction ($\delta^{15}\text{N}_{40}$), and $\delta^{15}\text{N}$ of >200 μm plankton size-
637 fraction ($\delta^{15}\text{N}_{200}$) in the latitudinal transects of 2007 and 2008 cruises. ** $p < 0.01$, $n=17$.

638 Table 3. Two-way factorial ANOVA (region, cruise) of $\delta^{15}\text{N}$ of suspended particles ($\delta^{15}\text{N}_{\text{sp}}$), $\delta^{15}\text{N}$
639 of 40-200 μm plankton size-fraction ($\delta^{15}\text{N}_{40}$), and $\delta^{15}\text{N}$ of >200 μm plankton size-fraction ($\delta^{15}\text{N}_{200}$)
640 in the 2007 and 2008 cruises. DF, degrees of freedom; SS, sums of squares; MS, mean of squares;
641 F, F statistic; p, probability.

642 Table 4. Mean \pm Standard deviation of the contribution of diazotroph nitrogen to $\delta^{15}\text{N}$ of suspended
643 particles, 40-200 μm plankton size-fraction ($\delta^{15}\text{N}_{40}$) and >200 μm plankton size-fraction ($\delta^{15}\text{N}_{200}$)
644 according to the two-end-member mixing model proposed by Montoya et al. (2002). The reference
645 zooplankton used in each fraction corresponded to the average of the stations sampled in the South
646 gyre during 2007, where *Trichodesmium* abundance was < 1 trichome L^{-1} , $\delta^{15}\text{N}_{40} = 4.6\text{‰}$, $\delta^{15}\text{N}_{200} =$
647 5.9‰ . The values of % of diazotroph N above 100 and below 0 were discarded in the calculation of
648 the regions average. Number of samples is indicated in parentheses.

649 Figure 1. Sampling stations during the TRYNITROP cruises on board the BIO 'Hespérides'. White
650 circles represent the autumn 2007 cruise (17 November - 8 December 2007), and grey triangles the
651 spring 2008 cruise (13 April - 2 May 2008).

652 Figure 2. Zonal and meridional vertical distribution of temperature ($^{\circ}\text{C}$), salinity and fluorescence in
653 autumn 2007 and spring 2008 cruises. Dashed lines in the temperature panels define the limits of
654 the three major regions identified by the depth of 16°C isotherm: North gyre, equatorial region and
655 South gyre.

656 Figure 3. Relationship between the $\delta^{15}\text{N}$ of suspended particles ($\delta^{15}\text{N}_{\text{sp}}$) and the particulate organic
657 nitrogen (PON) to chlorophyll *a* (chl-*a*) ratio during the autumn 2007 (a) and the spring 2008 (b)
658 cruises.

659 Figure 4. Zonal and meridional distributions of particulate organic nitrogen of suspended particles
660 (PON) during the autumn 2007 (a, b) and spring 2008 (c, d) cruises. Dashed lines define the limits
661 of the three major regions identified by the depth of 16°C isotherm in the meridional transect: North
662 gyre, equatorial region and South gyre. In the legend z1 to z6 represent the sampled depths from
663 deeper depth, z1 (DCM), to shallower depth, z6 (5m).

664 Figure 5. Vertical distribution of $\delta^{15}\text{N}$ of suspended particles ($\delta^{15}\text{N}_{\text{sp}}$) in autumn 2007 and spring
665 2008 cruises grouped by region: a, b) zonal transect ($15^\circ\text{-}38^\circ\text{ W}$), c, d) North gyre region (30°-
666 15°N), e, f) equatorial region ($15^\circ\text{N}\text{-}10^\circ\text{S}$), and g, h) South gyre region ($10^\circ\text{-}30^\circ\text{S}$).

667 Figure 6. Zonal and meridional distributions of the weighted mean of $\delta^{15}\text{N}$ of suspended particles
668 ($\delta^{15}\text{N}_{\text{sp}}$), the $\delta^{15}\text{N}$ of 40-200 μm plankton size-fraction ($\delta^{15}\text{N}_{40}$), the $\delta^{15}\text{N}$ of >200 μm plankton size-
669 fraction ($\delta^{15}\text{N}_{200}$), and concurrent measured N_2 fixation (Fernández et al., 2010; Fernández et al.,
670 2013) in autumn 2007 (a, b) and spring 2008 (c, d). Dashed lines define the limits of the three major
671 regions identified by the depth of 16°C isotherm in the meridional transect: North gyre, equatorial
672 region and South gyre.

673

674 Table 1.

	2007 cruise				2008 cruise			
	All stations	North gyre ^a	Equatorial region	South gyre	All stations	North gyre ^a	Equatorial region	South gyre
NH ₄	0.22* [128]	0.55* [54]	0.43** [38]	n.s.	n.s.	n.s.	0.47** [42]	-0.38* [36]
NO ₃	n.s.	0.30* [54]	n.s.	n.s.	0.21* [120]	n.s.	n.s.	0.51** [36]

675 ** p < 0.01, * p < 0.05, n.s. no significance

676 ^a includes the zonal and meridional legs in the North gyre.

677

678 Table 2.

	$\delta^{15}\text{N}_{\text{sp}}$		$\delta^{15}\text{N}_{40}$	
	2007	2008	2007	2008
$\delta^{15}\text{N}_{40}$	0.66 **	0.82 **	–	–
$\delta^{15}\text{N}_{200}$	0.66 **	0.74 **	0.90 **	0.78 **

679

680 Table 3.

681

		$\delta^{15}\text{N}_{\text{sp}}$				$\delta^{15}\text{N}_{40}$				$\delta^{15}\text{N}_{200}$			
	DF	SS	MS	F	p	SS	MS	F	p	SS	MS	F	p
region	2	23.6	11.8	6.3	0.004	26.6	13.3	11.5	0.000	25.7	12.8	15.4	0.000
cruise	1	16.0	16.0	8.6	0.006	4.8	4.8	4.2	0.048	15.9	15.9	19.2	0.000
region vs.cruise	2	6.0	3.0	1.6	0.213	20.9	10.5	9.0	0.000	21.7	10.9	13.1	0.000

682

683 Table 4.

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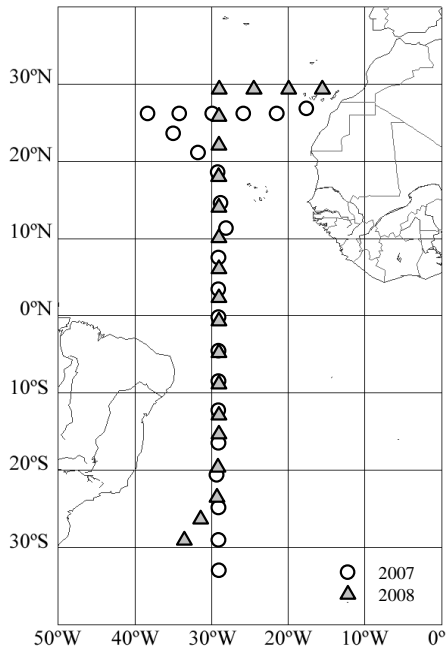
Region	Suspended particles		40-200 μ m zooplankton		>200 μ m zooplankton	
	2007	2008	2007	2008	2007	2008
Longitudinal transect	81 (29)	59 (3)	52 (14)	16 (9)	43 (4)	31 (6)
North gyre region	85 (14)	61 (10)	48 (22)	21 (11)	41 (12)	36 (8)
Equatorial region	62 (27)	39 (8)	25 (18)	3 (2)	11 (3)	29 (12)
South gyre region	49 (12)	58 (18)	15 (20)	36 (16)	13 (15)	40 (17)

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687 Figure 1.

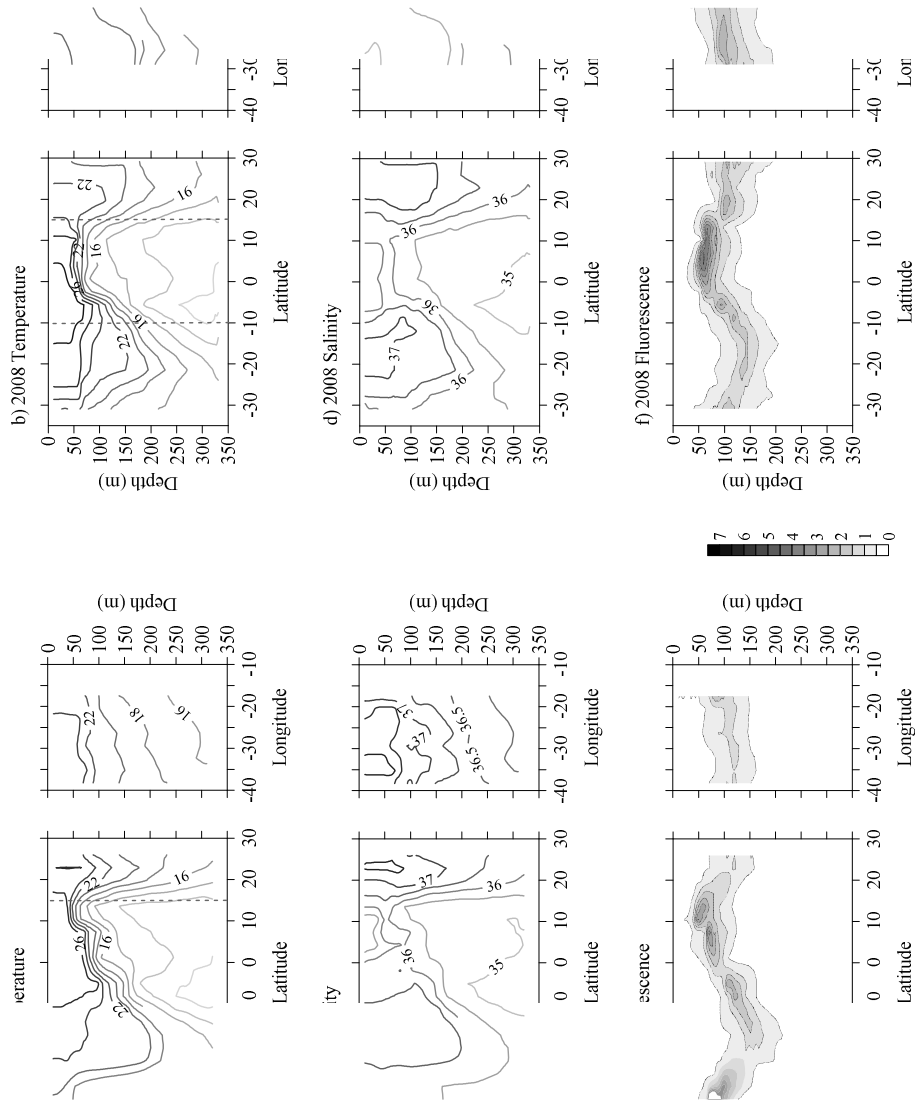
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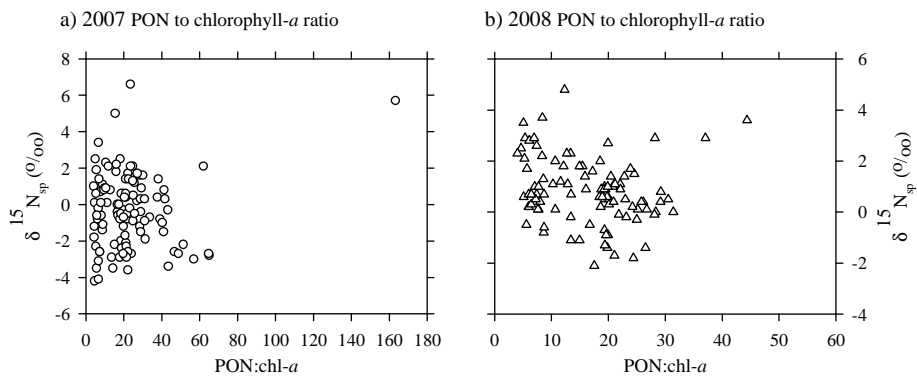


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694 Figure 3.

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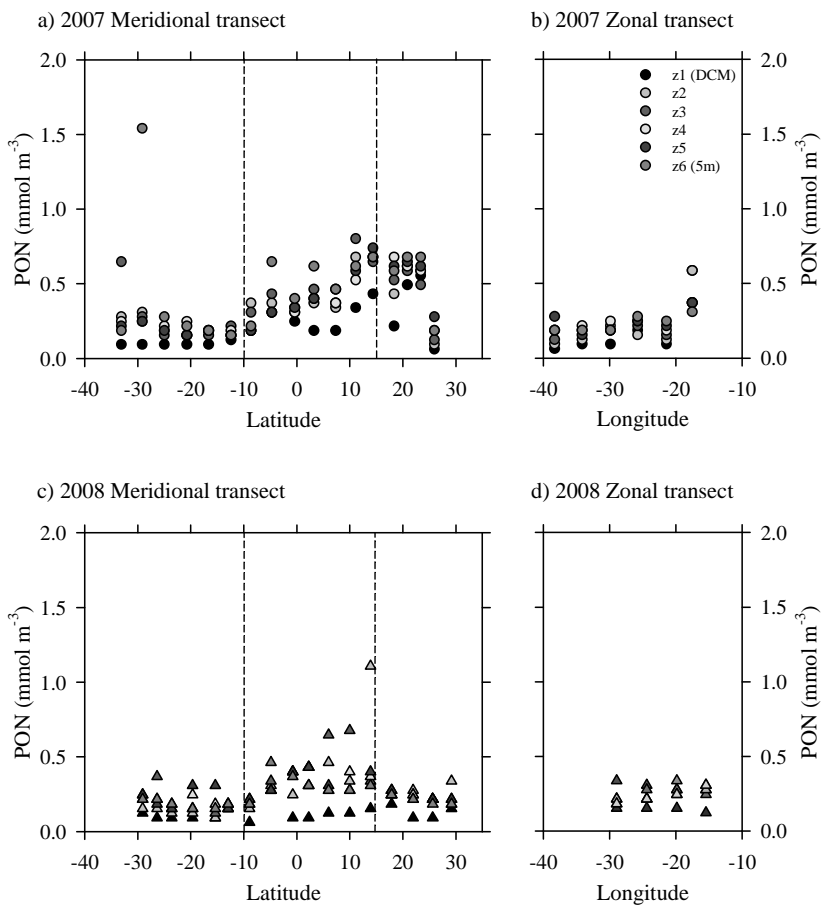


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698 Figure 4.

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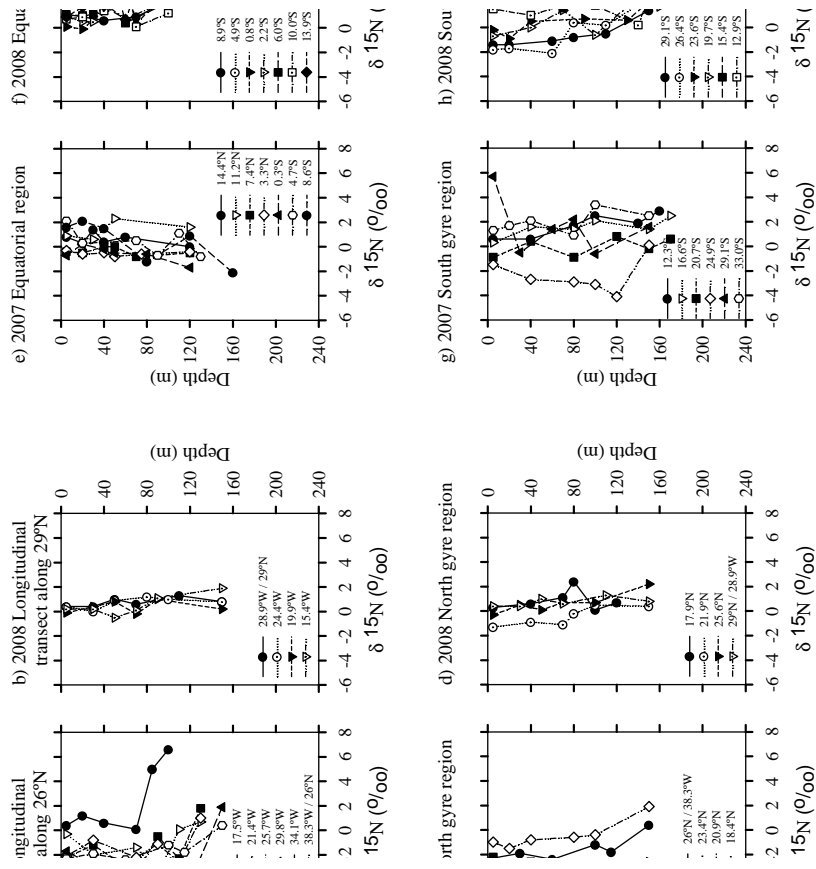


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702 Figure 5.

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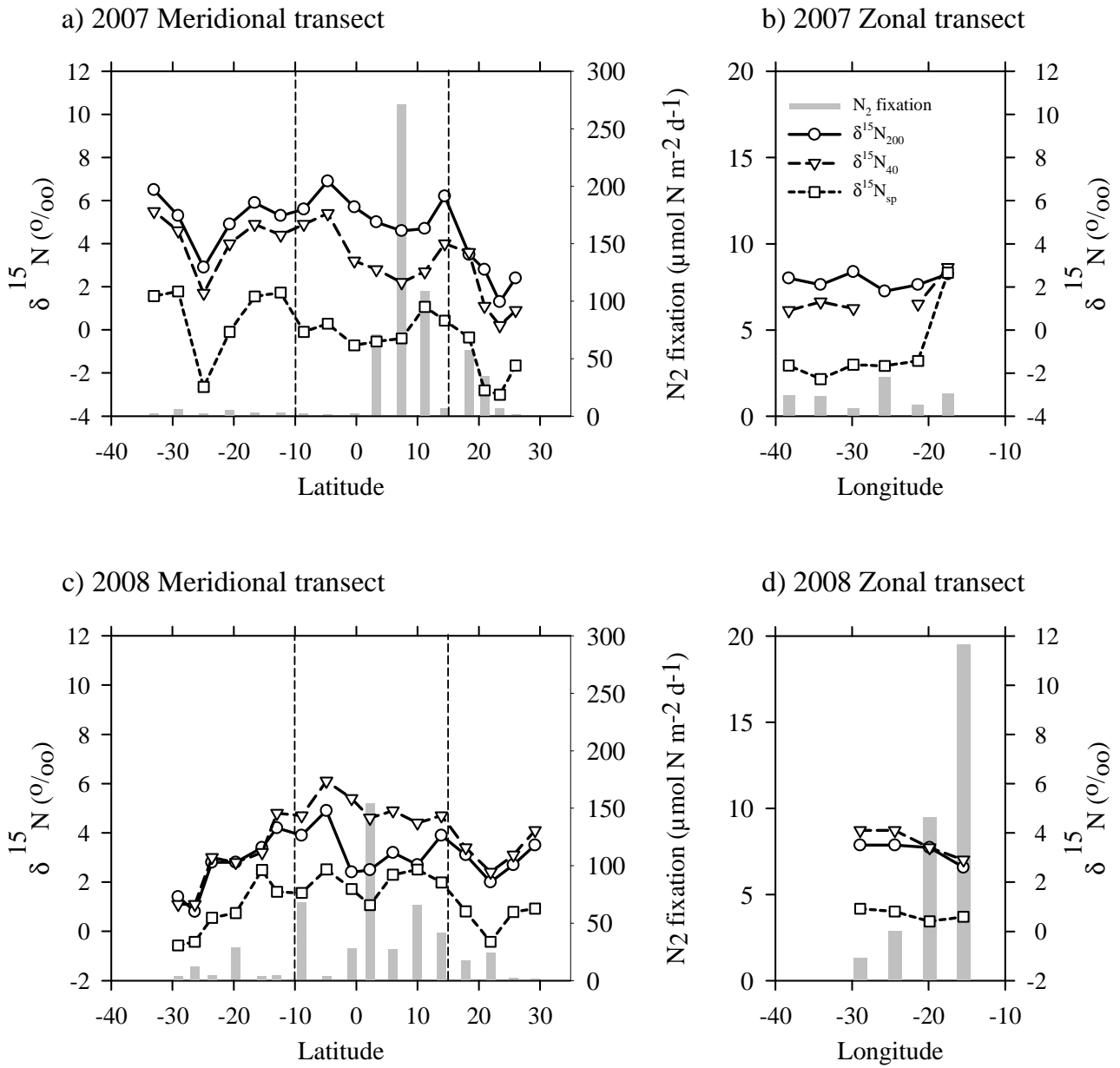


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706 Figure 6.

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