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Community N_2 fixation and *Trichodesmium* spp. abundance along longitudinal gradients in the eastern subtropical North Atlantic

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We have determined planktonic community N_2 fixation, *Trichodesmium* abundance, the concentration and vertical diffusive flux of phosphate, and satellite-derived estimates of atmospheric concentration of dust along two longitudinal transects in the eastern subtropical North Atlantic during November 2007 and from April–May 2008. *Trichodesmium* abundance was particularly low (<3 trichome l^{-1}) during the spring 2008 cruise, when low sea surface temperatures were recorded and vertical stratification was less marked. However, community N_2 fixation was always measurable, albeit low compared with other regions of the tropical Atlantic. The average, vertically-integrated N_2 fixation rate was 1.20 $\pm$ 0.48 μmol N m^{-2} d^{-1} in autumn 2007 and 8.31 $\pm$ 3.31 μmol N m^{-2} d^{-1} in spring 2008. The comparison of these rates of diazotrophy with the observed *Trichodesmium* abundances suggests that other, presumably unicellular, diazotrophs must have contributed significantly to community N_2 fixation, at least during the spring 2008 cruise. Satellite data of atmospheric dust concentration suggested similar rates of atmospheric deposition during the two surveys. In contrast, vertical diffusive fluxes of phosphate were 5-fold higher in spring than in autumn (14.2 $\pm$ 12.1 μmol P m^{-2} d^{-1} and 2.8 $\pm$ 2.6 μmol P m^{-2} d^{-1}, respectively), which may have stimulated N_2 fixation. These findings agree with the growing view that N_2 fixation is a more widespread process than the distribution of *Trichodesmium* alone may suggest. Our data also suggest a role for phosphorus supply in controlling the local variability of diazotrophic activity in a region subject to relatively high atmospheric inputs of iron.

Keywords: *Trichodesmium*, nitrogen fixation, eastern North Atlantic, diazotrophy.

Introduction

Nitrogen is the primary limiting nutrient for productivity in the world open ocean, and biological dinitrogen (N_2) fixation represents an important supply of new nitrogen to the euphotic layer in tropical and subtropical regions (Karl *et al.*, 2002). N_2 fixation can be more effective in sustaining net biological CO_2 drawdown than the vertical flux of nitrate, since the latter is also coupled with an upward flux of CO_2 from deeper waters (Michaels *et al.*, 2001). In the oceans, N_2 fixation is carried out by cyanobacteria and, within this group, the most studied species belong to the genus *Trichodesmium* (Capone *et al.*, 1997; Galloway *et al.*, 2004). Their ability to form large blooms, widespread distribution and high diazotrophic activity confer on them a relevant role in the global nitrogen cycle (Capone, 2001; Mahaffey *et al.*, 2005). *Trichodesmium* has been estimated to account for the fixation of 1.6×10^{12} mol N yr^{-1} in the tropical Atlantic Ocean (Capone *et al.*, 2005). However, recent studies point out that other organisms, mainly unicellular groups, may also be relevant (Zehr *et al.*, 2001; Montoya *et al.*, 2004; Moisander *et al.*, 2010). Some of these diazotrophic unicells distribute more uniformly in the water column (Langlois *et al.*, 2008) and their temperature optima for nitrogen fixation seem to be lower than that of

Trichodesmium (Langlois *et al.*, 2005; Stal, 2009). Measured rates of N_2 fixation attributable to unicellular fixers in those regions where they dominate the diazotrophic community are similar to those of regions with moderate or low abundance of *Trichodesmium* (Montoya *et al.*, 2004; Montoya *et al.*, 2007).

Relatively few studies have addressed the distribution and abundance of diazotrophs as well as their rates of N_2 fixation over large spatial scales (Moore *et al.*, 2009; Goebel *et al.*, 2010; Sohm *et al.*, 2011). This fact makes it difficult to define the physical, chemical and/or biological constraints that control diazotrophs distribution and activity and, therefore, hinders our understanding of these organisms and their impact in the nitrogen cycle. Predictive models are becoming useful tools for establishing large scale distributional patterns (Hood *et al.*, 2004; Monteiro *et al.*, 2010), but an extensive set of observations is still needed to validate these models.

The available studies suggest a westward increase in the abundance and N_2 fixation activity of *Trichodesmium* in the subtropical North Atlantic (Carpenter *et al.*, 2004; Montoya *et al.*, 2007; Goebel *et al.* 2010). Longitudinal changes in temperature, water column stability, upward diffusive fluxes of nutrients and atmospheric deposition of dust in the subtropical eastern North Atlantic are intriguing factors potentially affecting the distribution and activity of diazotrophs. This zonal variability has scarcely been studied in the eastern North Atlantic, in contrast to the thoroughly characterized western North Atlantic. To our knowledge, only the works of McCarthy and Carpenter (1979) and Benavides *et al.* (2011) provide a coupled study of the longitudinal variability of nitrogen fixation and *Trichodesmium* in this area of the ocean, complemented by González-Taboada *et al.* (2010), which gives a description of the variability of *Trichodesmium* in the water column.

The present work is part of a wider program investigating how different environmental gradients affect microbial community ecology and biochemical functioning in the tropical Atlantic (Marañón *et al.*, 2010; Huete-Ortega *et al.*, 2011; Moreno-Ostos *et al.*, 2011). We have previously reported on the latitudinal, large-scale variability in the factors that explain the asymmetric distribution of *Trichodesmium* and N_2 fixation in the North and South Atlantic Ocean (Fernández *et al.*, 2010). We have also analysed the latitudinal variability in the relative importance of nitrate eddy diffusion vs. N_2 fixation as sources of new nitrogen to the euphotic zone in the Atlantic Ocean (Mouriño-Carballido *et al.*, 2011). Here we focus on the eastern subtropical North Atlantic, starting from the hypothesis that longitudinal gradients in potential control constraints (i.e. temperature, water column stratification, and nutrient availability) can determine *Trichodesmium* abundance and distribution, as well as their importance for community N_2 fixation relative to that of unicellular fixers. Our main goal is to study the zonal variability in those controls during two contrasting seasons in the eastern subtropical North Atlantic and determine their possible role in controlling N_2 fixation in this region.

Methods

Sampling, hydrography and nutrients

Sampling took place 17–22 November 2007 and 29 April–2 May 2008 on board BIO "Hespérides" in the eastern North Atlantic Ocean (Figure 1), as part of the TRYNITROP (*Trichodesmium* and nitrogen fixation in the Atlantic Ocean) cruises. In both seasons, the ship's track followed a longitudinal transect, along 26°N from 17° to 38°W in 2007 and along 29°N from 15° to 29°W in 2008 comprising a total of 10 stations separated every ~ 400 km. Water sampling was carried out before dawn using 12-l Niskin bottles in a rosette which was cast to 300 m depth. Vertical distribution of temperature and salinity was measured by an SBE 911plus CTD (Conductivity, Temperature, Depth sensor) attached to the rosette. Samples to determine the vertical variability in nutrient concentration, chlorophyll *a* (Chl-*a*) concentration, particulate primary production and community $^{15}N_2$ fixation were taken at each station.

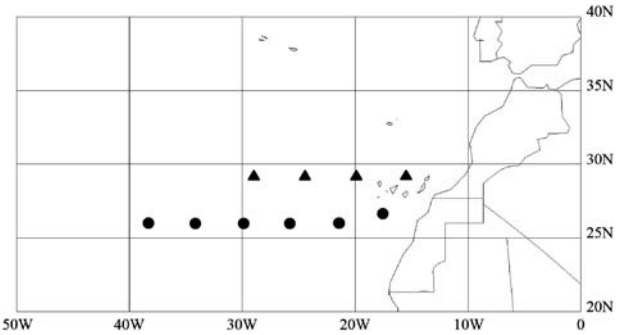

Figure 1. Map showing the stations of autumn 2007 (circles) and spring 2008 (triangles) longitudinal cruises, which took place 17–22 November 2007 and 29 April–2 May 2008, 41 × 22 mm (300 × 300 DPI).

Samples for inorganic phosphate from 12 depths (0–300 m) were stored frozen at −20°C until analysed at the on-shore laboratory, following standard colorimetric methods (Grasshof, 1976). The determination of nitrate, nitrite and ammonium concentration was performed on board on fresh samples using a Technicon segmented-flow auto-analyser and a modified colorimetric protocol which allows a detection limit of 2 nmol l^{-1} (Raimbault *et al.*, 1990; Kerouel and Aminot, 1997).

During the spring 2008 cruise, measurements of microstructure velocity shear were conducted down to 200 m using a microstructure profiler (Prandke and Stips, 1998) as described in Mouriño-Carballido *et al.* (2011). In this previous study the authors estimated the vertical diffusive fluxes of nitrate to the euphotic layer using the data retrieved by the microstructure profiler. Here we use these data to calculate the vertical fluxes of phosphate into the euphotic layer as the product of the estimated diffusion coefficients (kz) and the gradients of phosphate concentration, computed across 10 m depth steps by linear interpolation. Vertical diffusivity (kz) was estimated as in Osborn (1980):

$$kz = e\frac{\varepsilon}{N^2}$$

Where e is a mixing efficiency ($e = 0.2$), ε is the dissipation rate of turbulent kinetic energy and N is the Brunt-Väisälä frequency averaged over depth intervals of 10 m length for each station. Along the longitudinal section in spring 2008, the values of the measured dissipation rate of turbulent kinetic energy showed small variability between stations (−8.5 to −7.5 m^2 s^{-3}). Thus, we used the averaged vertical profile of ε values measured along 15–25°W in 2008 to compute the vertical diffusivity of phosphate in both the autumn 2007 and spring 2008 cruises.

Satellite-derived estimates of dust concentration

We used data available from the Giovanni online data system, developed and maintained by the NASA Goddard Earth Sciences (GES) Data and Information Services Center (DISC). In particular, we used Aqua-MODIS Aerosol Optical Depth at 550 nm and Aerosol Small Mode Fraction retrieved from a grid of 1° resolution and centred at the closest location to each station. These data, in combination with wind speed derived from AVISO (http://las.aviso.oceanobs.com/las/getUI.do) and the equations of Kaufman *et al.* (2005), were used to estimate the 15-day average atmospheric dust column concentration (g m^{-2}) at each station.

Chlorophyll *a*

For the measurement of Chl-*a* concentration, 6–7 depths were chosen using the fluorescence profile provided by the CTD in each station. 250-ml samples from each depth were filtered, using low vacuum pressure, through 0.2 μm pore-size polycarbonate filters. Extraction was made in 90% acetone overnight and fluorescence was measured on board afterwards with a Turner Designs 700 fluorometer, previously calibrated with pure chlorophyll *a* (Fluka).

Trichodesmium abundance

The ship's non-toxic water supply from ~ 5 m depth was used to determine the surface abundance of *Trichodesmium* trichomes. Samples were taken every 55–70 km along the transects. At each sampling point, from 60 to 115 litres of seawater were filtered through a 40-μm nylon mesh (15 cm in diameter). Particles were transferred by gentle rinsing of the mesh with filtered seawater (0.22 μm) to a 100-ml glass bottle, preserved in Lugol's solution and kept in darkness until counted. In order to evaluate the cellular integrity of the trichomes, samples collected both with the underway water supply and with Niskin bottles were regularly examined under the optical microscope. There were no differences in shape or length between bottle and non-toxic water supply samples. Besides, no broken or damaged filaments were found in any of the samples.

For the determination of the mean abundance of *Trichodesmium* spp. in the euphotic zone, samples were collected by vertical tows of a 40-μm net of 30 cm in diameter through the upper 200 m of the water column at each pre-dawn station. A flow meter was used to estimate the sampled volume. Then, samples were also transferred to 100-ml glass bottles and preserved in the same way as surface samples. Trichomes were counted under a Nikon Diaphot TMD microscope following the Utermöhl technique (Utermöhl, 1958).

Dinitrogen (N_2) fixation

N_2 fixation of the planktonic community was estimated using the $^{15}N_2$ technique of Montoya *et al.* (1996), as modified by Rees *et al.* (2009). At each station, three depths were sampled: surface (5 m), intermediate (30–80 m) and the deep chlorophyll maximum, DCM (50–150m). An initial 2-l sample for the determination of ^{15}N levels at time zero was taken at each depth and immediately filtered through a 25-mm diameter GF/F filter (Whatman). For the measurement of $^{15}N_2$ fixation, triplicate 2-l, acid-cleaned (10% diluted HCl), clear polycarbonate bottles (Nalgene) were filled at each depth, directly from the CTD-rosette. After removing all air bubbles, 2 ml of $^{15}N_2$ (98 atom%, SerCon) was injected into each bottle. The incubation was carried out on deck in a system of recirculating water simulating both *in situ* temperature, within a range of 2°C, and *in situ* photosynthetically active radiation levels, using a combination of blue and neutral density screens (061 Mist blue and 210 neutral density Lee filters, respectively). After 24 h, incubation was terminated by filtration of the entire sample through a 25-mm GF/F filter (Whatman). Afterwards all filters were dried at 40°C over 24 h and then stored until pelletization in tin capsules. Measurement of particulate organic nitrogen and ^{15}N atom% was carried out with an elemental analyser combined with a continuous-flow stable isotope mass-spectrometer (FlashEA112 + Deltaplus, ThermoFinnigan) and using an acetanilide standard as reference. The equations of Weiss (1970) and Montoya *et al.* (1996) were used to calculate the initial N_2 concentration (assuming equilibrium with the atmosphere) and N_2 fixation rates, respectively. The limit of detection for our rates was also estimated following the recommendations of Montoya *et al.* (1996). Using the time of incubation (1 day), the limit of detection of the analyser (0.20 μg N) and assuming that 4‰ is the minimum acceptable change in $\delta^{15}N$ of particulate organic nitrogen during the incubation, we found that the limit of detection was 0.001 μmol N m^{-3} d^{-1}.

A recent study has showed that $^{15}N_2$ fixation measurements using the injection of gas bubbles underestimates N_2 fixation significantly (Mohr *et al.*, 2010). However, these authors pointed out that the degree of this underestimation is not constant and varies with the incubation time, the volume of injected gas and the timing of the bubble injection relative to diel N_2 fixation. Here we incubated for a long period (24-h), which would have lead to a lower underestimation of the measure rates. Besides, no evidence suggests that this underestimation is systematically larger in any particular region of the oceans. Thus, the longitudinal patterns observed in this study are still valid, even if the absolute rates may be slightly underestimated.

Results

Hydrography, nutrients and atmospheric dust concentration

The hydrographic setting was markedly different in each cruise. In autumn 2007, upper layer temperatures were higher, reaching surface values >24°C during most of the transect (Figure 2a). This translated into a strong vertical stratification, as indicated by the vertical gradient in density from 5–200 m (Table 1), and the Brunt-Väisälä frequency vertical distribution in the euphotic zone (Figure 2c). In contrast, during spring 2008, surface waters were cooler (<20°C) (Figure 2b) and vertical stratification was weaker (Table 1, Figure 2d). In addition, during this last cruise the rise of the isotherms towards the East was more marked, reflecting a stronger influence of the North African upwelling. In particular, the 16°C isotherm rose from 290 m in the westernmost station to 220 m in the easternmost. This influence was also evident from the eastward rise of the nitracline depth (Table 1).

Similar values of phosphate concentration in the euphotic zone were measured in both cruises (Figure 3a and b). Mean concentrations of phosphate in the euphotic zone ranged from 0.02–0.04 μM and no apparent zonal trend was found in either cruise (Table 1). However, nitrate concentrations found in the euphotic zone were slightly higher in autumn 2007 than in spring 2008 (Figure 3c and d; Table 1). Also, no apparent trend was seen in the longitudinal distribution of mean nitrate in the euphotic zone during our surveys. By contrast, concentrations of both

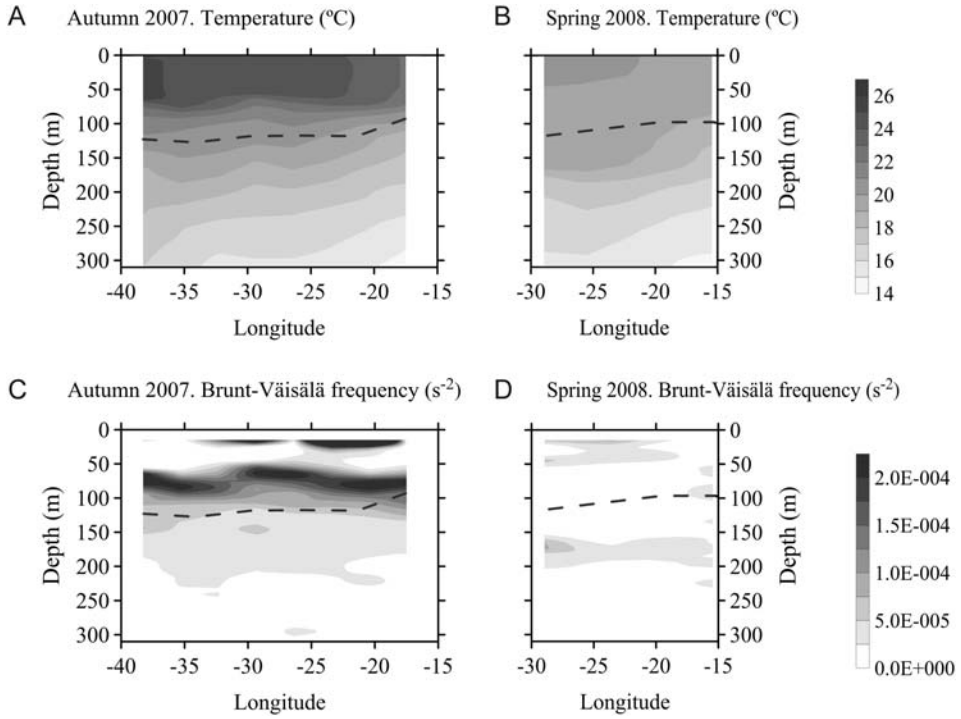

Figure 2. Longitudinal and vertical distribution of temperature (°C) and Brunt-Väisälä frequency (s^{-2}) during the autumn 2007 (left panels) and spring 2008 (right panels) surveys. Dashed lines represent the depth of the base of the euphotic zone (m), 114 × 90 mm (300 × 300 DPI).

Table 1. Physical, chemical and biological variables measured at each station during autumn 2007 and spring 2008 cruises on board B/O Hespérides in the eastern North Atlantic.

Date	Longitude	σ_t gradient [a] $\times 10^{-3}$ (kg m^{-4})	Nitracline depth[b](m)	Phosphocline depth[c](m)	$[NO_3]$ [d](nM)	$[PO_4]$ [d](μM)	Atmospheric dust (g m^{-2})	[chl-*a*][e]mg m^{-2}	Surface abundance of *Trichodesmium* (trichomes l^{-1})
17/11/2007	−17.49	6.16	109	150	84	0.04	0.61	26.9	0.5
18/11/2007	−21.38	6.42	117	130	104	0.02	0.80	28.3	0.5
19/11/2007	−25.73	7.18	86	150	118	0.02	0.68	29.1	0.4
20/11/2007	−29.83	6.35	116	200	102	0.02	0.22	22.6	2.6
21/11/2007	−34.12	5.32	99	150	61	0.02	0.13	23.8	7.4
22/11/2007	−38.26	7.40	96	150	63	0.03	0.23	23.6	6.9
02/05/2008	−15.45	1.89	100	100	57	0.03	0.70	23.3	1.6
01/05/2008	−19.87	2.10	136	180	43	0.04	0.70	19.2	1.6
30/04/2008	−24.41	2.25	154	180	60	0.03	0.37	21.3	0.6
29/04/2008	−28.94	2.65	230	180	28	0.02	0.27	23.2	1.1

[a]σ_t gradient is the density gradient between 5 and 200 m.
[b]Nitracline depth was estimated by interpolation and defined as the depth in which the concentration of nitrate reached 500 nM.
[c]Phosphocline depth was defined as the depth in which the concentration changed from a value of the order 0.0x to a value of the order 0.×
[d]$[NO_3]$ and $[PO_4]$ represent the mean concentration of nitrate and phosphate respectively in the euphotic zone.
[e][chl-*a*] is the vertically integrated total chlorophyll *a* from surface to the DCM depth.

nutrients below the euphotic zone were higher and depicted a stronger gradient in depth in spring 2008.

The estimated vertical diffusive fluxes of phosphate were markedly higher in 2008 (Figure 3e and f). The mean diffusive phosphate flux in a 50 m layer centred in the depth of the base of the euphotic zone was 5-fold higher in the spring 2008 cruise than in the autumn 2007 cruise (14.2 ± 12.1 μmol P m^{-2} d^{-1} and 2.8 ± 2.6 μmol P m^{-2} d^{-1}, respectively).

The average satellite-derived dust concentration in the atmosphere during both cruises increased to the east when approaching the coast of Africa and the Saharan desert. The estimated concentrations ranged between 0.13 and 0.80 g m^{-2} (Table 1). However, these values were relatively low in comparison with levels found during dust storm events.

Chlorophyll *a*

A similar distribution of Chl-*a* concentration was found in both cruises (Figure 4). Chl-*a* concentration was low in the upper mixed layer (<0.3 mg m^{-3}) and a distinct DCM was present at 100–120 m. The vertically integrated chl-*a* from the surface

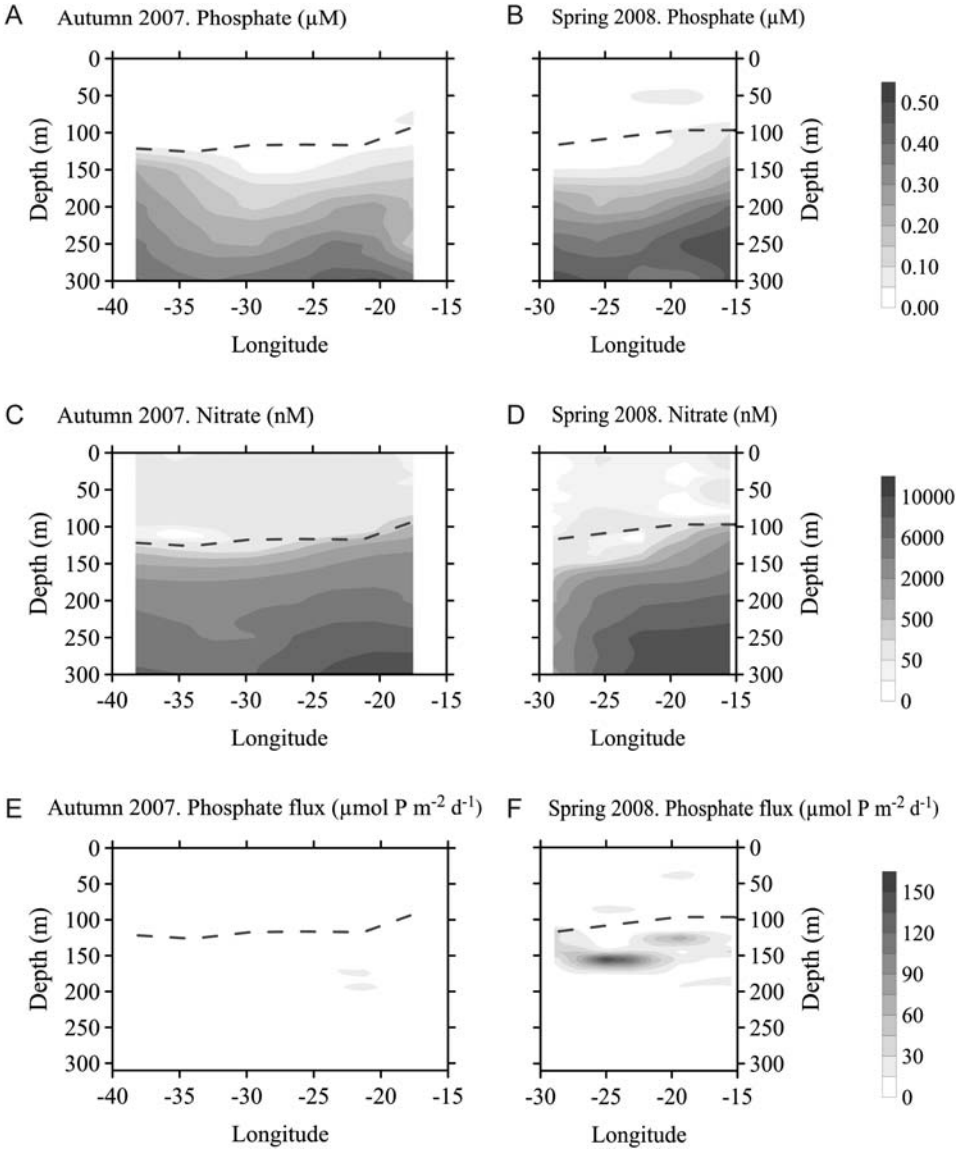

Figure 3. Longitudinal and vertical distribution of phosphate (μM), nitrate (nM) and vertical diffusive fluxes of phosphate (μmol P m^{-2} d^{-1}) computed across 10-m depth steps during autumn 2007 (left panels) and spring 2008 (right panels) cruises. Dashed lines represent the depth of the base of the euphotic zone (m), 177 × 221 mm (300 × 300 DPI).

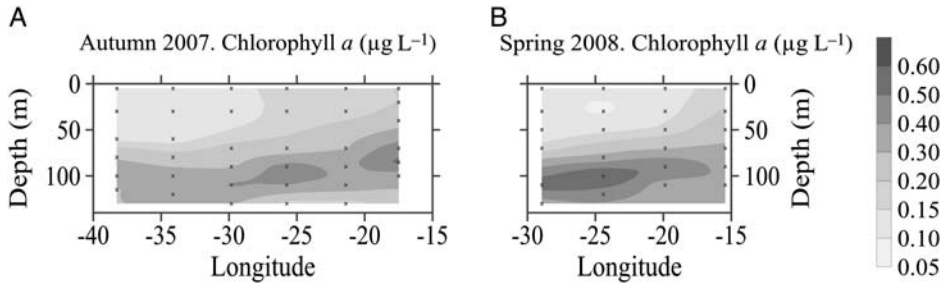

Figure 4. Longitudinal and vertical distribution of total chlorophyll *a* concentration (mg m^{-3}) in autumn 2007 (left panel) and spring 2008 (right panel) cruises. Crosses represent the sampled depths at each station, 49 × 16 mm (300 × 300 DPI).

down to the DCM depth ranged from 19 to 29 mg m^{-2} and showed little difference between cruises (25.7 $\pm$ 0.3 mg m^{-2} in 2007 vs. 21.9 $\pm$ 0.4 mg m^{-2} in 2008).

Trichodesmium abundance and community N_2 fixation

Only free trichomes were found in both transects. Less than 10 trichomes l^{-1} were measured, both in surface and euphotic zone samples, throughout the two transects with the exception of a slight increase at the surface to the west of 34°W during the 2007 survey (Figure 5a and b). Average surface abundances of *Trichodesmium* were 6.6 $\pm$ 13.5 and 0.8 $\pm$ 1.0 trichomes l^{-1} in the autumn 2007 and spring 2008 surveys, respectively.

The rates of surface community N_2 fixation were very low but always above the limit of detection during 2007 (Figure 5c). Measured rates in 2008 were up to 10-fold higher (Figure 5d) although always below 0.3 μmol N m^{-3} d^{-1}.

No clear longitudinal trend was detected in surface diazotroph activity throughout the transects (Figure 5c and d). However, when considering N_2 fixation rates in the water column down to the DCM, a clear eastward trend in rates at intermediate depth and DCM was present in spring 2008 (Figure 6a), with an increasing contribution of intermediate depth communities to the overall activity in the euphotic zone. Besides, in the easternmost station of this cruise (15.45°W), the rates measured at 50 and 90 m clearly exceeded that of surface waters. The vertically integrated diazotrophic activity measured during autumn 2007 was always low (<2.3 μmol N m^{-2} d^{-1}), with no apparent longitudinal trend along the transect (Figure 6b). The average areal rate on this cruise was 1.2 $\pm$ 0.5 μmol N m^{-2} d^{-1}. In contrast, spring 2008 areal nitrogen fixation depicted a clear increasing trend towards the east (Figure 6b) reaching a maximum of 20 μmol N m^{-2} d^{-1} at the easternmost station, where high concentrations of atmospheric dust were also derived from satellite data. The average, vertically integrated N_2 fixation rate on this cruise was 8.3 $\pm$ 3.3 μmol N m^{-2} d^{-1}, a value 7-fold higher than that of autumn 2007.

Discussion

Trichodesmium distribution

The zonal distribution of *Trichodesmium* in the North Atlantic Ocean has been reported to be asymmetric with higher abundances and more frequent bloom occurrence in the western side (Carpenter *et al.*, 2004; Montoya *et al.*, 2007). Our results agree with these findings, as *Trichodesmium* were almost absent in surface waters during our cruises in the eastern North Atlantic in two contrasting seasons, and only a moderate westward increase of *Trichodesmium* was found on the autumn 2007 cruise. However, the environmental control factors involved in this zonal distribution remain unclear (Montoya *et al.*, 2007).

Temperature is an important constraint for *Trichodesmium* distribution (Karl *et al.*, 2002). Studies with cultures have shown that it grows in a range between 20°C and 34°C, but the optimum for N_2 fixation and growth lies between 24°C and 30°C (Breitbarth *et al.*, 2007). In addition, Stal (2009) suggests that at temperatures below 20°C non-heterocystous diazotrophic cyanobacteria such as

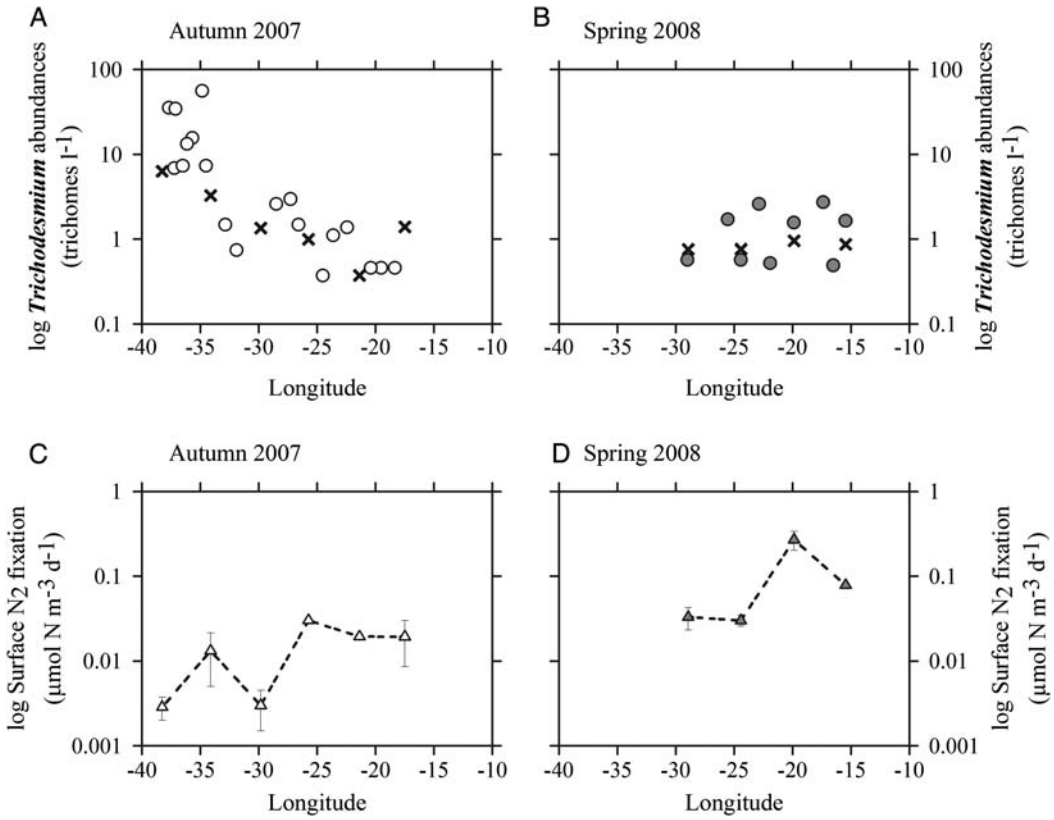

Figure 5. Longitudinal distribution of *Trichodesmium* abundance (trichomes l^{-1}) and mean surface community N_2 fixation (μmol N m^{-3} d^{-1}) during autumn 2007 (left panels, white symbols) and spring 2008 (right panels, grey symbols). In the upper panels, crosses represent the abundance of *Trichodesmium* as estimated from the net tows (0–200 m) and full symbols represent surface (~5 m depth) abundance. Bars in the N_2 fixation plots represent the standard deviation of the mean (n=3), 123 × 95 mm (300 × 300 DPI).

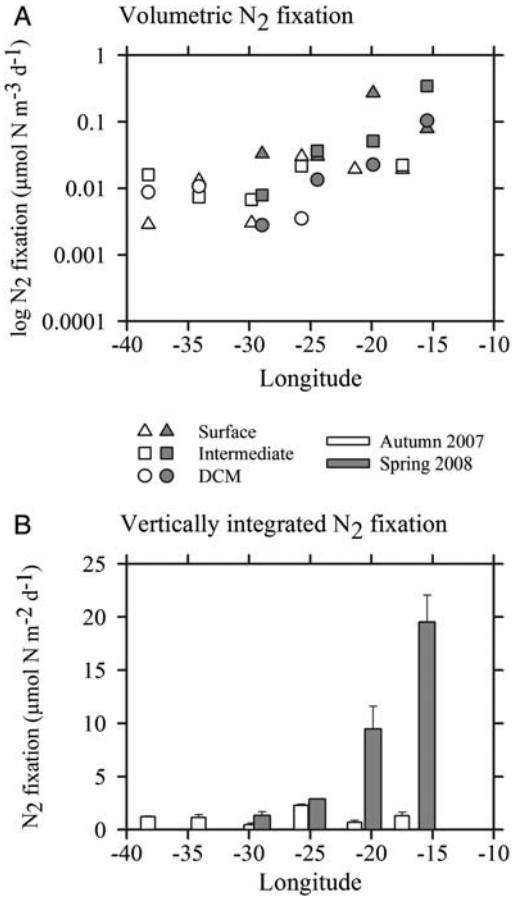

Figure 6. Longitudinal distribution of volumetric N_2 fixation (µmol N m^{-3} d^{-1}) at each depth sampled (upper panel) and vertically integrated N_2 fixation (µmol N m^{-2} d^{-1}) at each station (lower panel) in autumn 2007 (white symbols) and spring 2008 (grey symbols) cruises. Bars in the vertically integrated N_2 fixation plot represent the standard deviation of the mean ($n = 3$), 176 × 310 mm (300 × 300 DPI).

Trichodesmium cannot maintain the local anoxic conditions required by nitrogenase to remain active and therefore they tend to be excluded from waters below that temperature. Therefore, temperature would explain the near absence of trichomes in the water column during the spring 2008 cruise, when temperatures below 20–21°C were recorded. Other physical factors that may favour *Trichodesmium* include the presence of a shallow upper mixed layer and a high stability of the water column (LaRoche and Breitbarth, 2005). In autumn 2007, the upper mixed layer was relatively shallow, with the beginning of the thermocline located at ~ 75 m (Figure 2a), and the water column was more stable than in spring 2008, as indicated by the higher values of both the gradient of σ_t and the Brunt-Väisälä frequency (Table 1, Figure 2c). Thus, the combination of all these factors, together with the westward increasing trend in water temperature, would explain the higher *Trichodesmium* abundances observed during autumn 2007.

Nitrogen fixation

In contrast to the intensively studied and thoroughly characterized, in terms of diazotrophy, western North Atlantic, available data on the longitudinal variability of N_2 fixation rates in the subtropical eastern North Atlantic are still scarce. In our area of study, McCarthy and Carpenter (1979) investigated the longitudinal variability in N_2 fixation by *Trichodesmium* colonies collected with nets in surface waters in May–June 1975. Their measurements were done by the acetylene reduction assay in 2-h incubations at midday. Thus, in order to compare the results, we transformed these rates using their measured abundances of cells and assuming an average of 150 cells per trichome and 10 h per day of N_2 fixation by *Trichodesmium* (Mulholland *et al.*, 2006). In addition, the work of Benavides *et al.* (2011), provides an evaluation of community diazotrophic activity in surface waters from the results of the acetylene reduction assay, the $^{15}N_2$ technique and also the activity due only to *Trichodesmium* in the euphotic zone as measured by the acetylene reduction assay. However, it is important to remember when comparing all these measurements that the acetylene reduction assay is an indicator of gross nitrogen fixation, whereas the $^{15}N_2$ technique is a measure of net nitrogen fixation (Montoya *et al.*, 1996). The gross vertically-integrated activities measured by McCarthy and Carpenter (1979) were always < 2 µmol N m^{-2} d^{-1}, whereas Benavides *et al.* (2011) obtained *Trichodesmium* gross nitrogen fixation rates that were even lower (<0.1 µmol N m^{-2} d^{-1}), albeit similar to our rates of autumn 2007, when the water column temperature was more suitable for *Trichodesmium*. Even though the abundance of this cyanobacterium in these previous two studies and in our study was very low (<10 trichomes l^{-1}), it seems that *Trichodesmium* can sustain modest but significant rates of N_2 fixation in this region of the North Atlantic Ocean.

No statistical correlation was found between the surface distribution of *Trichodesmium* and the measured N_2 fixation at 5 m. With the purpose of assessing if *Trichodesmium* could sustain the measured surface community N_2 fixation in our two cruises, we used the large dataset of *in situ* measurements collected by Mulholland *et al.* (2006) to calculate the 25th and 75th percentiles of the distribution of N_2 fixation rate per trichome (0.007 and 0.030 nmol N trichome^{-1} d^{-1}, respectively). These, in combination with our measured *Trichodesmium* surface abundances, were used to estimate a likely upper and lower limit for *Trichodesmium*-based N_2 fixation in each station. The upper limit would represent the maximum N_2 fixation rate by *Trichodesmium* in favourable conditions, whereas the lower limit would be the minimum fixation rate expected in each station. In autumn 2007, all measured surface community N_2 fixation rates were below the corresponding upper limit, which would suggest that *Trichodesmium* could account for the observed diazotrophic activity. However, the surface rates measured in spring 2008 (0.03–0.27 µmol N m^{-3} d^{-1}) were always higher than the upper estimated limit in each station, which ranged between 0.02–0.05 µmol N m^{-3} d^{-1}. Therefore, the surface community N_2 fixation during the spring 2008 cruise is unlikely to have been fully sustained by *Trichodesmium*. The same pattern is depicted when applying this approach to the comparison of the vertically-integrated N_2 fixation and the abundance of *Trichodesmium* collected by nets (Figure 5a and b) and integrated to the DCM depth, with the single exception of the most western station surface waters in the spring 2008 cruise, where, as happened in autumn 2007, the N_2 fixation in the euphotic layer could be explained by *Trichodesmium*.

Lower water column temperatures observed in the 2008 survey may have favoured the presence of unicellular diazotrophs (e.g. unicellular group A or group B), which can occur at lower

temperatures than *Trichodesmium* and tend to be distributed throughout the water column rather than concentrated near the surface (Langlois *et al.*, 2005). Diazotrophic activity in intermediate waters in spring 2008 was similar to that measured at the surface, and the maximum rate was found at 50 m in the easternmost station of this cruise. Thus, the vertical distribution of N_2 fixation rates observed in spring 2008 would be consistent with the view that unicellular species dominated the diazotrophic activity during this cruise. Besides, the magnitude of the highest rates on this cruise is similar to those previously reported in other oceanic regions where unicellular diazotrophs dominated the community (Goebel *et al.*, 2010; Moisander *et al.*, 2010). These observations, together with our previous measurements in the South Atlantic subtropical gyre (Fernández *et al.*, 2010), support the growing view that other diazotrophs, in addition to *Trichodesmium*, are important players in the nitrogen cycle in surface oceanic waters (Moisander *et al.*, 2010).

Nitrogen fixation can be limited by the availability of both iron and phosphorous (Capone, 2001; Karl *et al.*, 2002). Recent studies suggest that iron supply through atmospheric deposition is the key factor controlling the large-scale distribution of diazotrophy in the Atlantic Ocean (Moore *et al.*, 2009; Fernández *et al.*, 2010). The experimental addition of Saharan dust has been shown to stimulate N_2 fixation in the tropical and subtropical Atlantic (Mills *et al.*, 2004; Marañón *et al.*, 2010). Our region of study is subject to high atmospheric dust deposition due to its proximity to the Saharan desert, and shows relatively high dissolved iron concentrations (Moore *et al.*, 2009). In this regard, Sañudo-Wilhelmy *et al.* (2001) showed that during a cruise to the central Atlantic Ocean, when background iron concentrations were relatively high, N_2 fixation by *Trichodesmium* was independent of iron concentration and, instead, correlated strongly with the phosphorus content of the colonies. We thus consider the possibility that differences in phosphorus supply may have contributed to explain the inter-cruise differences in N_2 fixation observed during our study. Atmospheric dust is the main source of iron for the surface ocean, but may supply phosphorus as well (Ridame and Guieu, 2002; Mills *et al.*, 2004). The atmospheric concentration of dust was similar and described the same eastward trend during the two cruises (Table 1). Therefore, we would expect a similar amount of atmospheric deposition of both iron and phosphorus in the autumn 2007 and spring 2008 cruises. On the other hand, phosphorus is also supplied to the euphotic zone through vertical diffusive fluxes from below the thermocline. The estimated vertical diffusive fluxes of phosphate in spring 2008 clearly exceeded those found in autumn 2007; this difference may explain the higher rates of diazotrophy observed in the former cruise. In addition, the exclusion of *Trichodesmium* by cooler water temperatures would have reduced the competition for phosphate among diazotrophs, thus allowing the small unicellular cyanobacterial groups to actively fix N_2. Whether or not phosphorus supply plays a role in controlling the zonal variability of N_2 fixation in the NE subtropical Atlantic will only be ascertained unequivocally with data on the intracellular content of phosphorous in diazotrophs, so further studies are still required.

Conclusions

Our study contributes to and further expands on the relatively small dataset of available measurements of N_2 fixation levels and *Trichodesmium* abundance in the eastern subtropical North Atlantic. Temperature and vertical stability conditions appeared as key factors explaining the distribution of *Trichodesmium*, which showed particularly low abundances during spring 2008, when the water column was colder and less strongly stratified. Our results indicate that N_2 fixation is always measurable in the region even when *Trichodesmium* is present in very low abundance. Moreover, the comparison of measured N_2 fixation rate with the concurrent *Trichodesmium* abundance points to the role of other diazotrophs, particularly when sea temperatures are colder. Finally, the contrasting magnitude of the vertical diffusive flux of phosphate in each cruise suggests a role for phosphorus as a controlling factor of the local distribution of N_2 fixation (Sañudo-Wilhelmy *et al.*, 2001) in a region that receives relatively high amounts of iron through atmospheric deposition (Moore *et al.*, 2009).

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