Evidence of high N\textsubscript{2} fixation rates in productive waters of the temperate Northeast Atlantic

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Abstract. Diazotrophic activity and primary production (PP) were investigated along two transects (Belgica BG2014/14 and GEOVIDE cruises) off the western Iberian Margin and the Bay of Biscay (38.8°–46.5° N; 8.0°–19.7° W) in May 2014 close to the end of the spring bloom. We report substantial N\textsubscript{2} fixation activities, reaching up to 65 nmol N L\textsuperscript{-1} d\textsuperscript{-1} and 1533 µmol N m\textsuperscript{-2} d\textsuperscript{-1} close to the Iberian Margin between 38.8° N and 40.7° N. Similar figures in the basin have only been reported in the temperate and tropical western North Atlantic waters with coastal, shelf or mesohaline characteristics, as opposed to the mostly open ocean conditions studied here. In agreement with previous studies, the qualitative assessment of \textit{nifH} gene diversity (encoding the nitrogenase enzyme that fixes N\textsubscript{2}) suggested a predominance of heterotrophic diazotrophs, and the absence of filamentous cyanobacteria. At the sites where N\textsubscript{2} fixation activity was highest sequences affiliated to UCYN-A1, obligate symbiont of eukaryotic prymnesiophyte algae, were recovered. The remaining phylotypes were non-cyanobacterial diazotrophs, known to live in association with suspended particles and zooplankton (i.e., Bacteroidetes, Firmicutes and Proteobacteria). Outside the area of exceptional activity, N\textsubscript{2} fixation in the open ocean and at shelf-influenced sites was also relatively high, ranging from 81 to 384 µmol N m\textsuperscript{-2} d\textsuperscript{-1}, but was undetectable in the central Bay of Biscay. We propose that the unexpectedly high heterotrophic N\textsubscript{2} fixation activity recorded at the time of our study was sustained by the availability of phytoplankton derived organic matter (dissolved and/or particulate) resulting from the ongoing to post spring bloom. We pose that this organic material not only sustained bacterial production, but also provided sufficient nutrients essential for the nitrogenase activity (e.g., phosphorus). Dissolved Fe was supplied through atmospheric dust deposition during the month preceding our study and through advection of surface waters from the subtropical region and the shelf area. Our findings stress the need for a more
detailed monitoring of the spatial and temporal distribution of oceanic N$_2$ fixation in productive waters of the temperate North Atlantic to better constrain the basin-scale nitrogen input to the ocean inventory.

### 1 Introduction

Dinitrogen (N$_2$) fixation is the major pathway of nitrogen (N) input to the global ocean and thereby contributes to sustaining oceanic primary productivity (Falkowski, 1997). The conversion by N$_2$-fixing micro-organisms (diazotrophs) of dissolved N$_2$ gas into bioavailable nitrogen also contributes to euphotic layer new production and as such, to the sequestration of atmospheric carbon dioxide into the deep ocean (Gruber, 2008). Estimating the overall contribution of N$_2$ fixation to carbon sequestration in the ocean requires an assessment of the global marine N$_2$ fixation which is to date a matter of debate (Luo et al., 2012).

In tropical and subtropical regions, surface waters characterized by warm, stratified and depleted dissolved inorganic nitrogen (DIN) conditions, are assumed to give a competitive advantage to diazotrophs over other phytoplankton since only they can draw N from the unlimited dissolved N$_2$ pool for their biosynthesis. The filamentous cyanobacterium Trichodesmium, long considered as the most active diazotroph in the global ocean, has mostly been reported from oligotrophic tropical and subtropical regions of the ocean (Dore et al., 2002; Capone et al., 2005; Montoya et al., 2007; Needoba et al., 2007; Moore et al., 2009; Fernández et al., 2010; Snow et al., 2015), thought to represent the optimal environment for its growth and N$_2$-fixing activity (Capone, 1997; Breithbarth et al., 2007). As such, past estimates of global annual N$_2$ fixation were mainly based on information gathered from tropical and subtropical regions (Luo et al., 2012). However, it was recently suggested that Trichodesmium might also be abundant in temperate waters of the Atlantic (Benavides and Voss, 2015; Rivero-Calle et al., 2016) and Pacific Oceans (Shiozaki et al., 2015), even though these higher latitude areas have been poorly explored for diazotrophic activity. Studies using genetic approaches targeting genes encoding the nitrogenase enzyme that fixes N$_2$ (e.g. *nifH*), have shown the existence and importance of other diazotrophic organisms which apparently occupy broader ecological niches (Sohm et al., 2011; Zehr, 2011). Small diazotrophs such as unicellular diazotrophic cyanobacteria (UCYN classified in groups A, B and C) and non-cyanobacterial diazotrophs, mostly heterotrophic bacteria (e.g. Alpha and Gammaproteobacteria), have been observed over a wide depth range and latitudinal scale, thus spanning a broad range of temperatures (Langlois et al., 2005, 2008; Krupke et al., 2014; Cabello et al., 2015).

In the Northeast Atlantic, the large input of Saharan iron-rich dust alleviating dissolved iron (dFe) limitation of the nitrogenase activity (Fe being a co-factor of the N$_2$-fixing enzyme) (Raven, 1988; Howard and Rees, 1996), and the upwelling of subsurface waters with low DIN (dissolved inorganic nitrogen) to phosphate ratios (Deutsch et al., 2007; Moore et al., 2009), make this region highly favorable for N$_2$ fixation activity. In fact, the tropical and subtropical eastern North Atlantic waters have been reported to harbor a particularly diverse diazotrophic community relative to the western border and other basins (Langlois et al., 2008; Zehr, 2011; Ratten et al., 2015). The temperate eastern North Atlantic has even been observed to be a worldwide hotspot of prymnesiophyte-UCYN-A symbiotic associations (Cabello et al., 2015).

The discovery of a methodological bias associated to the commonly used $^{15}$N$_2$ bubble-addition technique (Mohr et al., 2010), and the presence of an abundant diazotrophic community in high latitude regions actively fixing N$_2$ (Needoba et al., 2007; Rees et al., 2009; Blais et al., 2012; Mulholland et al., 2012; Shiozaki et al., 2015) indicate that more efforts are
needed to better constrain oceanic N\textsubscript{2} fixation and diazotrophic diversity. Earlier studies in the Iberian Basin investigated the diazotrophic activity either during stratified water column conditions of boreal summer and autumn (Moore et al., 2009; Benavides et al., 2011; Snow et al., 2015; Fonseca-Batista et al., 2017) or during winter convection period (Rijkenberg et al., 2011; Agawin et al., 2014). In the present work, we provide evidence for the significance of N\textsubscript{2} fixation, based on the \textsuperscript{15}N\textsubscript{2} dissolution method and examine the \textit{nifH} diversity in the Iberian Basin under ongoing and post spring bloom conditions.

2 Material and Methods

Field experiments were conducted during two nearly simultaneous cruises in May 2014. The Belgica BG2014/14 cruise (21–30 May 2014, R/V Belgica), investigated the Bay of Biscay and the western Iberian Margin. In parallel, the GEOVIDE expedition in the framework of the international GEOTRACES program (GA01 section, May 16 to June 29 2014, R/V “Pourquoi pas?”) sailed from Portugal shelf area towards Greenland and ended in Newfoundland, Canada (http://dx.doi.org/10.17600/14000200). For the latter expedition, only stations within the Iberian Basin investigated for N\textsubscript{2} fixation activity (stations Geo-1, 2, 13 and 21) are considered in this paper and the measurements are compared with those conducted at the six sites studied during the BG2014/14 cruise (stations Bel-3, 5, 7, 9, 11 and 13; Figure 1).

2.1 Environmental conditions

Temperature, salinity and photosynthetically active radiation (PAR) profiles were determined using a conductivity-temperature-depth sensor (SBE 09 and SBE 911+, during the BG2014/14 and GEOVIDE cruises, respectively) fitted on a rosette equipped with either 12 or 24 Niskin bottles to sample seawater for biogeochemical measurements. Water column concentrations of ammonium (NH\textsubscript{4}\textsuperscript{+}), during both cruises were measured on board as well as nitrate + nitrite (NO\textsubscript{3}\textsuperscript{–} + NO\textsubscript{2}\textsuperscript{–}) concentrations during the GEOVIDE expedition. During the BG2014/14 cruise, samples dedicated for NO\textsubscript{3}\textsuperscript{–} + NO\textsubscript{2}\textsuperscript{–} and phosphate (PO\textsubscript{4}\textsuperscript{3–}) measurements were filtered (0.2 µm) and stored at –20°C until analyses at the home-based laboratory. PO\textsubscript{4}\textsuperscript{3–} data are not yet available for the GEOVIDE cruise.

Nutrient concentrations were determined using conventional fluorometric (for NH\textsubscript{4}\textsuperscript{+}) (Holmes et al., 1999) and colorimetric methods (for all others) (Grasshoff et al., 1983) with detection limits (DL) of 64 nmol L\textsuperscript{–1} (NH\textsubscript{4}\textsuperscript{+}), 90 nmol L\textsuperscript{–1} (NO\textsubscript{3}\textsuperscript{–} + NO\textsubscript{2}\textsuperscript{–}) and 60 nmol L\textsuperscript{–1} (PO\textsubscript{4}\textsuperscript{3–}). For the BG2014/14 cruise, chlorophyll \textit{a} (Chl \textit{a}) concentrations were determined according to Yentsch and Menzel (1963), by filtering 250 mL of seawater sample onto Whatman GF/F glass fiber filters (0.7 µm nominal pore size), followed by pigment extraction in 90% acetone, centrifugation and fluorescence measurement using a Shimadzu RF-150 fluorometer. For the GEOVIDE cruise, chlorophyll and carotenoid pigments were determined as described in Ras et al. (2008): 2.3 L of seawater samples were filtered onto Whatman GF/F glass fiber filters, followed by extraction in 100% methanol, disrupted by sonication, clarified by filtration (Whatman GF/F) and analyzed by High-performance liquid chromatography (HPLC, Agilent Technologies system).
2.2 $^{15}$N$_2$ fixation and $^{13}$C-HCO$_3^-$ uptake rates

$N_2$ fixation and primary production (PP) were determined simultaneously in duplicate using the $^{15}$N-$N_2$ dissolution method (Großkopf et al., 2012) and $^{13}$C-NaHCO$_3$ tracer addition (Hama et al., 1983) techniques. Seawater samples were collected in 4.5 L acid-cleaned polycarbonate (PC) bottles from a minimum of four depths (six at stations Geo-1, Geo-13 and Geo-21) equivalent to 54%, 13%, 3% and 0.2% of surface PAR (plus 25% and 1% PAR for the 3 exceptions). Details concerning the applied $^{15}$N$_2$ dissolution method can be found in Fonseca-Batista et al. (2017). Briefly, $^{15}$N$_2$-enriched seawater was prepared by degassing prefiltered (0.2 µm) seawater, thereafter stored in 2 L gastight Tedlar bags (Sigma-Aldrich) subsequently injected with 30 mL of pure $^{15}$N$_2$ gas (98 $^{15}$N atom%, Eurisotop, lot number 23/051301) and left to equilibrate. This $^{15}$N$_2$ gas batch (Eurisotop) has previously been shown to be free of $^{15}$N-labelled contaminants such as nitrate, nitrite, ammonium and nitrous oxide. Each incubation PC bottle was partially filled with sampled seawater, then amended with 250 mL of $^{15}$N-enriched seawater, spiked with 3 mL of a NaH$^{13}$CO$_3$ solution (200 mmol L$^{-1}$, 99%, Eurisotop) and topped off with the original seawater sample. Samples were incubated for 24 hours in on-deck incubators circulated with surface seawater and wrapped with neutral density screens (Rosco) simulating the in situ irradiance conditions. After incubation, samples were filtered onto pre-combusted MGF filters (glass microfiber filters, 0.7 µm, Sartorius), which were subsequently dried at 60°C and stored at room temperature. The natural concentration and isotopic composition of particulate organic carbon and particulate nitrogen (POC and PN) were assessed by filtering an additional 4.5 L of non-spiked seawater from each depth. All samples were measured for POC and PN concentrations and isotopic compositions using an elemental analyzer (EuroVector Euro EA 3000) coupled to an isotope mass spectrometer (Delta V Plus, Thermo Scientific) and calibrated against international certified reference material (CRM): IAEA-N1 and IAEA-305B for N and IAEA-CH6 and IAEA-309B for C. $N_2$ fixation and carbon uptake volumetric rates were computed as described in Montoya et al. (1996), and depth-integrated rates were calculated by non-uniform gridding trapezoidal integration for each station. Minimal detectable uptake rates were determined as detailed in Fonseca-Batista et al. (2017). To do so, the minimal acceptable $^{15}$N or $^{13}$C enrichment of PN or POC after incubation (Montoya et al., 1996) is considered to be equal to the natural isotopic composition, specific to each sampled depth, increased by three times the uncertainty obtained for N and C isotopic analysis of CRM. All remaining experiment-specific terms are then used to recalculate the minimum detectable uptake. Carbon uptake rates were always above their specific DL, while $N_2$ fixation was undetectable for some incubations, see details in section 3.3.

2.3 DNA sampling and nifH diversity analysis

During the BG2014/14 cruise, water samples used for $^{15}$N$_2$ and NaH$^{13}$CO$_3$ incubations were also collected for DNA extraction prior to incubation. 2 L volumes were vacuum filtered (20 to 30 kPa) through 0.2 µm sterile cellulose acetate filters (47 mm Sartorius type 111) subsequently placed in cryovials directly flash deep frozen in liquid nitrogen. At the land-based laboratory, samples were transferred to a −80°C freezer until nucleic acid extraction. DNA was extracted from the samples using the Power Water DNA Isolation kit (MOBio) and checked for integrity by agarose gel electrophoresis. The amplification of nifH sequences was performed on 3–50 ng µL$^{-1}$ environmental DNA samples using one unit of Taq polymerase (SPrIME), by nested PCR according to Zani et al. (2000) and Langlois et al. (2005). Amplicons of the predicted 359-bp size observed by gel electrophoresis were cloned using the PGEM T Easy cloning kit (PROMEGA) according to the manufacturer’s instructions. A total of 103 clones were sequenced by the Sanger technique.
150 (GATC, Marseille). DNA alignments were performed using the Molecular Evolutionary Genetics Analysis software
151 (MEGA 7.0) (Kumar et al., 2016) and nifH operational taxonomic units (nifH-OTUs) were defined with a 5% divergence
cutoff. DNA sequences were translated into amino acid sequences, then nifH evolutionary distances which are considered
153 as the number of amino acid substitutions per site, were computed using the Poisson correction method (Nei, 1987). All
154 positions containing gaps and missing data were eliminated (see phylogenetic tree in Supporting Information Figure S1).
155 One sequence of each nifH-OTU was deposited in GenBank under the accession numbers referenced from KY579322 to
156 KY579337.

158 3 Results

159 3.1 Ambient environmental settings

160 Sites sampled in May 2014 during the Belgica BG2014/14 and GEOVIDE cruises, were located within the Iberian Basin
161 Portugal Current System (PCS) (Ambar and Fiúza, 1994) which is influenced by highly fluctuating wind stress (Frouin et
162 al., 1990).
163 The predominant upper layer water mass in this basin is the Eastern North Atlantic Central Water (ENACW), a winter
164 mode water which consists of two components according to Fiúza (1984) (see 0/S diagrams, Figure 2): (i) the lighter,
165 relatively warm and salty ENACWst formed in the subtropical Azores Front region (~35° N) when Azores Mode Water is
166 subducted as a result of strong evaporation and winter cooling; and (ii) the colder and less saline ENACWsp, underlying
167 the ENACWst, and formed in the subpolar eastern North Atlantic (north of 43° N) through winter cooling and deep
168 convection (McCartney and Talley, 1982). The spatial distribution of these Central Waters allowed categorizing the
169 sampling sites in 2 groups: (i) ENACWsp stations north of 43° N (Bel-3, Bel-5, Bel-7, and Geo-21) only affected by the
170 ENACWsp (Figures 2a and 2b) and (ii) ENACWst stations, south of 43° N, characterized by the upper layer being
171 influenced by ENACWst and the subsurface layer by ENACWsp (Figures 2a and 2b). Some of these ENACWst stations
172 are open ocean sites (Bel-9, Bel-11, Bel-13, and Geo-13) while others are shelf-influenced (Geo-1 and Geo-2) (Tonnard
173 et al., 2018).
174 Surface waters of all the ENACWst stations showed a relatively strong stratification resulting from the progressive spring
175 heating, with sea surface temperature (SST) ranging from 15.3 (Geo-13) to 17.2°C (Bel-13). Nutrients were depleted at
176 the surface (NO$_3^-$ + NO$_2^-$ < 0.09 µM in the upper 20 m; Figures 3c and 3f) and surface Chl a concentrations were low (<
177 0.25 µg L$^{-1}$; Figures 3a and 3d) but showed a subsurface maximum (between 0.5 and 0.75 µg L$^{-1}$ at approximately 50 m),
a common feature for oligotrophic open ocean waters. Amongst the ENACWst stations, station Geo-13 had a slightly
178 higher nutrient content (NO$_3^-$ + NO$_2^-$ = 0.7 µM in the lower mixed layer depth, MLD) and higher Chl a (> 0.5 µg L$^{-1}$ in
179 the upper 35 m).
180 Surface waters at ENACWsp stations were less stratified (SST between 14.0 and 14.5°C), were nutrient replete (surface
181 NO$_3^-$ + NO$_2^-$ ranging from 0.3 to 0.8 µM) and had a higher phytoplankton biomass (Chl a between 0.7 to 1.2 µg L$^{-1}$ in the
182 upper 30 m except for station Bel-5). Highest Chl a values were observed at station Bel-7 (44.6° N, 9.3° W), which
183 appeared to be located within an anticyclonic mesoscale eddy, as evidenced by the downwelling structure detected in the
184 Chl a and NO$_3^-$ + NO$_2^-$ profiles (Figures 3a and 3c) at this location (as well as T and S sections, data not shown).
3.2 Primary production and pigment distribution

Volumetric rates of carbon uptake ranged from 7 to 3500 µmol C m$^{-3}$ d$^{-1}$ (see Supporting Information Table S1) and euphotic layer integrated rates from 32 to 137 mmol C m$^{-2}$ d$^{-1}$ (Figure 4 and Supporting Information Table S2). PP was relatively homogenous in the Bay of Biscay (stations Bel-3, Bel-5 and Bel-7) and along the Iberian Margin (Bel-9, Bel-11, Bel-13 and Geo-1) with average rates ranging from 33 to 43 mmol C m$^{-2}$ d$^{-1}$, except at station Bel-7 where it was slightly higher (52 mmol C m$^{-2}$ d$^{-1}$; Figure 4 and Supporting Information Table S2), likely due to the presence of an anticyclonic mesoscale structure at this location. PP increased westwards away from the Iberian Peninsula, reaching highest values at stations Geo-13 and Geo-21 (79 to 135 mmol C m$^{-2}$ d$^{-1}$, respectively; Figure 4) as well as closer to the shelf (reaching 59 mmol C m$^{-2}$ d$^{-1}$ at Geo-2). These results are in the range of past measurements for the same period of the year, ranging from 19 to 103 mmol C m$^{-2}$ d$^{-1}$ (Marañón et al., 2000; Fernández et al., 2005; Poulton et al., 2006). Our observations also coincide with the area-averaged Chl a time series obtained from satellite data (from the Giovanni online data system; Figure 5) which reveal that post bloom conditions prevailed at most sites (Bel-3 to Bel-13 and Geo-1 to Geo-13) while the bloom was still ongoing at station Geo-21 at the time of our study. Higher PP rates appear to coincide with the increase, offshore and towards the shelf, of the relative abundance of diatoms, based on fucoxanthin pigment concentrations (Figure 6) (Tonnard et al., in preparation for this Special issue). At the less nutrient replete GEOVIDE sites Geo-1 and Geo-13, prymnesiophytes represented 30–40% of the phytoplankton community, compared to 20–35% at stations Geo-21 and Geo-2 (based on the presence of 19'-hexanoyloxyfucoxanthin pigment). Such relative abundances are in agreement with the global abundance of prymnesiophytes (32 ± 5%) proposed by Swan et al. (2016).

3.3 Diazotrophic activity and diversity

Where detectable, volumetric N$_2$ fixation rates ranged from 0.7 to 65.4 nmol N L$^{-1}$ d$^{-1}$ (see Supporting Information Table S1), with areal rates ranging between 81 and 1533 µmol N m$^{-2}$ d$^{-1}$ (Figure 7 and Supporting Information Table S2). We observed very high N$_2$ fixation activities at the two sites (Bel-11 and Bel-13) most affected by ENACW waters of subtropical origin (Figure 2). There, volumetric rates of N$_2$ fixation ranged from 2.4 to 65.4 nmol N L$^{-1}$ d$^{-1}$ and average areal rates from 1355 to 1533 µmol N m$^{-2}$ d$^{-1}$. We were able to recover from the BG2014/14 cruise 103 nifH sequences (from positive PCR amplifications) in surface waters (54% PAR level) of stations Bel-11 and Bel-13. No successful nifH amplifications were obtained at other stations and depths where diazotrophic activities were lower. At station Bel-11, a single OTU was recovered as all nifH sequenced clones (n = 41) had 99% similarity at the nucleotide level and 100% similarity at the amino acid level with Candidatus Atelocyanobacterium thalassa isolate (heterotrophic diazotrophic cyanobacteria, UCYN-A1; Figures 8A and S1) from station ALOHA in the North Pacific (Thompson et al., 2012). Station Bel-13 illustrated an apparent greater diversity, as indicated by the 15 OTUs recovered there (from 62 sequenced clones; Figures 8A–B and S1) and their percentage contribution to the Chao 1 estimates of total nifH-OTUs reaching 17.5 for this station. The latter species richness index gives an estimate of total number of species for a given community, based on the number of singleton (OTU represented by a single read) and doubletons (OTU obtained twice) found in the sample (Chao, 1984). This suggests that for Bel-13, recovered OTUs could explain 85.7% of the diversity. Among these 15 OTUs, 45.2% of the sequences were affiliated to UCYN-A1 (identical to those found at Bel-11), and the rest to heterotrophic bacteria with 25.8% affiliated to Bacteroidetes, 19.3% to Firmicutes and 9.7% to Proteobacteria (Figures 8A and S1).
Shelf-influenced (Geo-1 and Geo-2) and open ocean (Geo-13) ENACWst sites, besides Bel-11 and Bel-13, displayed relatively high N\(_2\) fixation activities with volumetric rates ranging between 1.0 and 7.1 nmol N L\(^{-1}\) d\(^{-1}\) (Supporting Information Table S1) and average depth-integrated rates of 141, 262 and 384 µmol N m\(^{-2}\) d\(^{-1}\), respectively (Figure 7 and Supporting Information Table S2). N\(_2\) fixation was also high at the most productive ENACWsp sites Bel-7 and Geo-21 with volumetric rates ranging from 1.0 to 8.2 nmol N L\(^{-1}\) d\(^{-1}\) and average areal rates of 128 and 279 µmol N m\(^{-2}\) d\(^{-1}\), respectively. However, no diazotrophic activity was measured at ENACWsp sites Bel-3 and Bel-5 in the central Bay of Biscay nor at specific depths of stations Bel-9 (120 m), Bel-11 (45 m) and Geo-21 (18 m).

We computed the relative contribution of N\(_2\) fixation to PP by converting N\(_2\) fixation rates to carbon uptake using either a Redfield ratio of 6.6 or the determined median POC/PN ratio for natural particles (equivalent to the mean value of 6.3 ± 1.1, ± SD, n = 46; Table 1). N\(_2\) fixation contributed to less than 2% of PP in the ENACWsp sites and between 3 to 28% of PP in the southernmost ENACWst sites, except at station Bel-9 where it supported about 1% of PP. These contributions reach values twice as high as those reported in other studies for the oligotrophic tropical and subtropical eastern Atlantic usually considered as systems prone to host diazotrophic activity (contributions to PP ranging from < 1% to 12%) (Voss et al., 2004; Rijkenberg et al., 2011; Fonseca-Batista et al., 2017). However, it is important to keep in mind that this computation relies on the assumption that only photoautotrophic diazotrophs contribute to bulk N\(_2\) fixation, which is not always the case, particularly in the present study.

4 Discussion

During two expeditions to the Iberian Basin and Bay of Biscay in May 2014 (38.8–46.5° N), we observed N\(_2\) fixation activity in surface waters of most stations (except at the two northernmost sites in the Bay of Biscay), characterized by relatively low SST (12.5–17.3°C) and a wide range of DIN concentrations (NO\(_3^-\) + NO\(_2^-\) from < 0.1 to 7.6 µM). In the following sections we discuss (1) the major contributors to diazotrophic activity in these high latitude regions, (2) the significance of N\(_2\) fixation in the Iberian Basin, and to a wider extent in the whole Atlantic, and (3) the potential environmental drivers of the unexpectedly high diazotrophic activity in the Iberian Basin.

4.1 Features of the diazotrophic community composition in the temperate North Atlantic

Diazotrophic diversity investigation during the Belgica BG2014/14 cruise (stations Bel-3 to Bel-13) revealed the presence of nifH sequences only in the surface waters of ENACWst stations Bel-11 and Bel-13, where we observed the highest areal N\(_2\) fixation rates, exceeding 1000 µmol N m\(^{-2}\) d\(^{-1}\) (Figures 7 and 8). Our qualitative assessment of nifH diversity suggested a predominant role of heterotrophic diazotrophs (UCYN-A as the singular cyanobacterial phylotype and non-cyanobacterial diazotrophs) and the lack of presence of Trichodesmium filaments. These findings are corroborated by the more quantitative assessment of nifH diversity carried out at stations Geo-1, Geo-2, Geo-13 and Geo-21 (Julie LaRoche, personal communication, 2018) confirming that only heterotrophic non-cyanobacterial phytootypes contributed to the observed N\(_2\) fixation activity. For the BG2014/14 cruise, UCYN-A1 represented 67% of all nifH sequences recovered off the Iberian Margin, with the remainder sequences belonging to non-cyanobacterial groups (Bacteroidetes, Firmicutes and Proteobacteria). Previous work in temperate regions of the global ocean (Needoba et al., 2007; Rees et al., 2009;...
Mulholland et al., 2012; Shiozaki et al., 2015) including the Iberian Margin (Agawin et al., 2014; Moreira-Coello et al., 2017) also reported that highest N\textsubscript{2} fixation activities were predominantly related to the presence of UCYN-A cells (UCYN-A1, UCYN-A2 and UCYN-A3 clades being only identified a posteriori) (Thompson et al., 2014) and heterotrophic bacteria, while *Trichodesmium* filaments were low or undetectable.

UCYN-A cells (in particular from the UCYN-A1 clade) were shown to live in mutualistic symbioses with single-celled prymnesiophyte algae (Thompson et al., 2012). This symbiotic association was believed to be obligate since UCYN-A are characterized by an unusually streamlined genome lacking essential cyanobacterial features such as the photosystem II, the RuBisCo and the tricarboxylic acid cycle (Zehr et al., 2008; Tripp et al., 2010). The prymnesiophyte-UCYN-A1 symbiosis was consistently observed during the work of Cabello et al. (2015) across the global ocean and is thus being considered as an obligate association. In addition, this symbiosis has been reported to be particularly abundant in the central and eastern basin of the North Atlantic (Krupke et al., 2014; Cabello et al., 2015), which is consistent with the fact that a relatively important proportion of prymnesiophyte species were observed in that region during the GEOVIDE cruise (Figure 6).

Bacteroidetes, commonly encountered in the marine environment, are considered as specialized degraders of organic matter that preferably grow attached to particles or algal cells (Fernández-Gómez et al., 2013). Studies have shown the presence of N\textsubscript{2} fixation and/or nitrogenase-like genes (among which *nifH* and/or *nifD*) in the genome of several species of this phylum (Dos Santos et al., 2012; Inoue et al., 2015). Furthermore, N\textsubscript{2} fixation activity has been reported in five Bacteroidetes strains including *Bacteroides graminisolvens*, *Paludibacter propionicigenes* and *Dysgonomonas gadei* (Inoue et al., 2015) which are the closest cultured relatives of the *nifH*-OTUs detected at station Bel-13 (Figure S1). The remaining sequences were affiliated to Cluster III phylotypes of functional nitrogenase genes, which mainly consist of anaerobic bacteria containing molybdenum nitrogenase genes such as *Clostridium* (Firmicutes), *Desulfovibrio* (Deltaproteobacteria), *Sulfurospirillum* (Epsilonproteobacteria) (Chien and Zinder, 1996). Anaerobic Cluster III phylotypes have been previously recovered from different ocean basins (Church et al., 2005; Langlois et al., 2005, 2008; Man-Aharonovich et al., 2007; Rees et al., 2009; Halm et al., 2012; Mulholland et al., 2012). These diazotrophs were suggested to benefit from anoxic microzones found within marine snow particles or zooplankton guts to fix N\textsubscript{2} thereby avoiding oxygenic inhibition of their nitrogenase enzyme (Braun et al., 1999; Church et al., 2005; Scavotto et al., 2015). Therefore, the bloom to early post bloom conditions prevailing during our study, probably constituted an ideal condition for diazotrophic groups that depend on the availability of detrital organic matter availability or association with grazing zooplankton.

No *Trichodesmium* filaments were recovered during the BG2014/14 cruise, nor during GEOVIDE sampling in the Iberian region, although *Trichodesmium* spp. have recently been reported to be widely distributed in temperate waters of the North Atlantic (Rivero-Calle et al., 2016). Despite the fact that our sampling strategy (Niskin sampling) is not suited for a quantitative recovery of *Trichodesmium* (Montoya et al., 2007), it is likely that any presence of filaments would still have been detected had they been present at the time of our field investigation. This was also confirmed by a CHEMTAX analysis of pigments (Tonnard et al., in preparation for this Special issue).

Our findings further support the important role played by small diazotrophs, particularly heterotrophic groups, in introducing new N to the oceanic budget. These observations tend to comfort the idea of a substantial role played not only by UCYN-A (Cabello et al., 2015; Martínez-Pérez et al., 2016) but also by non-cyanobacteria (Halm et al., 2012;
4.2 Significance of N₂ fixation in the temperate ocean

In the present study, we found surprisingly high N₂ fixation activities at most of the studied sites. Rates were exceptionally elevated at two open ocean sites located between 38.8°–40.7° N at about 11° W (averaging 1533 and 1355 µmol N m⁻² d⁻¹ at stations Bel-11 and Bel-13, respectively; Figure 7 and Tables S1 and S2). Although N₂ fixation was not detected in the central Bay of Biscay (stations Bel-3 and Bel-5), rates recorded at all the other sites were relatively high, not only in shelf-influenced areas (141 and 262 µmol N m⁻² d⁻¹ at stations Geo-1 and Geo-2, respectively) but also in the open ocean (average activities between 81–384 µmol N m⁻² d⁻¹ at stations Bel-7, Bel-9, Geo-13 and Geo-21).

Previous studies in the Iberian Basin (35°–50° N, east of 25° W) reported relatively lower N₂ fixation rates (from < 0.1 to 140 µmol N m⁻² d⁻¹), regardless of whether the bubble-addition method of Montoya et al. (1996) or the dissolution method by Mohr et al. (2010) and Groskopf et al. (2012) were used. However, these studies were carried out largely outside the bloom period, either during the late growth season (summer and autumn) (Moore et al., 2009; Benavides et al., 2011; Snow et al., 2015; Riou et al., 2016; Fonseca-Batista et al., 2017) or during winter (Rijkenberg et al., 2011; Agawin et al., 2014). In contrast, the present study took place in spring, during or just at the end of the vernal phytoplankton bloom. Differences in timing of these different studies and to a lesser extent, differences in applied methodologies (bubble-addition relative to the dissolution method) may explain the discrepancies in diazotrophic activity observed between our study and earlier works.

Our values are either similar or up to one order of magnitude higher than maximal N₂ fixation rates reported for the eastern tropical and subtropical North Atlantic further south (reaching up to 360–424 µmol N m⁻² d⁻¹) (Groskopf et al., 2012; Subramaniam et al., 2013; Fonseca-Batista et al., 2017). Yet, conditions favouring N₂ fixation are commonly believed to be met in these tropical and subtropical regions of the North Atlantic: (1) intense Saharan dust deposition providing dissolved iron (dFe), a co-factor of the nitrogenase enzyme (Raven, 1988; Howard and Rees, 1996); (2) stronger stratification (resulting in DIN-depleted surface waters) (Capone et al., 2005; Luo et al., 2014) and (3) input via eastern boundary upwelling of subsurface waters carrying excess of PO₄³⁻ relative to NO₃⁻ (i.e., excess relative to the canonical Redfield P/N ratio; expressed as P*). This positive P* signature in subsurface waters of the Atlantic Ocean is considered to originate either from the Indo-Pacific (Deutsch et al., 2007; Moore et al., 2009; Ratten et al., 2015; Fonseca Batista et al., 2017) or the Arctic (Yamamoto-Kawai et al., 2006).

In the Atlantic Ocean, very high N₂ fixation rates up to ~1000 µmol N m⁻² d⁻¹ as observed here, have only been reported for temperate coastal waters of the Northwest Atlantic (up to 838 µmol N m⁻² d⁻¹) (Mulholland et al., 2012) and for tropical shelf-influenced and mesohaline waters of the Caribbean and Amazon River plume (maximal rates ranging between 898 and 1600 µmol N m⁻² d⁻¹) (Capone et al., 2005; Montoya et al., 2007; Subramaniam et al., 2008). Shelf and mesohaline areas have indeed been shown to harbor considerable N₂ fixation activity, not only in tropical regions (Montoya et al., 2007; Subramaniam et al. 2008) but also in areas from temperate to polar regions (Rees et al., 2009; Blais et al., 2012; Mulholland et al., 2012; Shiozaki et al., 2015). Yet, the environmental features which enhance diazotrophic activity in these regions are not fully understood. For tropical mesohaline systems the conditions proposed to drive such an intense diazotrophic activity include the occurrence of highly competitive symbiosis, i.e. diatom-
diazotrophs associations, besides the influence of a positive P* input from the Amazon River (Subramaniam et al., 2008). However, such conditions of excess P were however not observed in the present study (see section 4.3) nor in previous studies carried out in high latitude shelf regions with elevated N₂ fixation activities (Blais et al., 2012; Mulholland et al., 2012; Shiozaki et al., 2015). In addition, while tropical mesohaline regions are characterized by the predominance of diatom-diazotroph associations (and filamentous Trichodesmium spp.), in temperate shelf areas the diazotrophic community is reported to be essentially dominated by heterotrophic diazotrophs, from UCYN-A symbionts of prymnesiophyte algae to Proteobacteria and Cluster III phylotypes (Rees et al., 2009; Blais et al., 2012; Mulholland et al., 2012; Agawin et al., 2014; Shiozaki et al., 2015).

We propose that bloom-related processes are partly responsible for the unprecedented high N₂ fixation rates observed in the Iberian region at the time of our study. In the next section, we investigate environmental drivers which could, in combination with the latter, explain the large range of N₂ fixation rates observed in the present study (from undetectable rates up to 1533 µmol N m⁻² d⁻¹).

4.3 Key environmental drivers of N₂ fixation

In the past decades, the study of environmental factors regulating marine N₂ fixation has specifically focused on autotrophic cyanobacteria as these were generally believed to be the most active diazotrophs (Zehr et al., 2001; Luo et al., 2014). Nevertheless, the commonly recognized environmental controls such as solar radiation, sea surface temperature, DIN and dissolved oxygen concentration (Luo et al., 2014), may not directly affect the heterotrophic diazotrophs. Indeed by having fundamentally different ecologies relative to autotrophic diazotrophs, heterotrophic diazotrophs are expected to exhibit discrete regulation of their N₂ fixation activity (Bombar et al., 2016). Nonetheless, molecular and cellular conditions for sustaining N₂ fixation activity and related processes still requires the supply of DFe and P (Raven, 1988; Howard and Rees, 1996). DFe and P availability (Mills et al., 2004; Moore et al., 2009) as well as positive P* signature (Deutsch et al., 2007; Moore et al., 2009; Ratten et al., 2015; Fonseca-Batista et al., 2017) are in fact considered to regulate the distribution of bulk oceanic N₂ fixation. Besides these critical inorganic nutrients, heterotrophic N₂ fixation was also recently shown to be highly dependent on the availability of organic matter (Bonnet et al., 2013; Rahav et al., 2013, 2016; Loescher et al., 2014).

In the present study we hypothesize that seasonality of phytoplankton production is a major driver of N₂ fixation in the Iberian Margin. Since surface waters at the time of our study were under bloom to post-bloom conditions, they likely carried significant amounts of organic matter which may have promoted the growth of heterotrophic diazotrophs. This hypothesis is supported by findings from the GEOVIDE cruise showing that surface waters of the Iberian Basin (stations Geo-1 and Geo-13) and the West European Basin (Geo-21) illustrated rather variable but significant upper column (100–120 m) particulate organic carbon concentration (POC of 166, 171 and 411 mmol C m⁻², respectively) with a dominant fraction of small size POC (1–53 µm) relative to total abundance (75%, 92% and 64%, respectively) (Lemaître et al., 2018). Small cells, usually being slow-sinking particles, are considered easily remineralized in surface waters (Villa-Alfageme et al., 2016) which was confirmed by the very low export efficiency observed at stations Geo-13 and Geo-21 (3% and 4% of euphotic layer integrated PP reaching the depth of export, respectively) evidencing an efficient shallow remineralisation (due to bacterial and zooplankton activity). Although the upper 100 m at station Geo-1 was characterized by a less effective recycling of organic matter (export efficiency of 35%), likely due to lithogenic ballast effect and to the
presence of larger and/or denser phytoplankton groups, export efficiency there may have been overestimated to an unusually low in situ PP (relative to satellite-based estimates) (Lemaitre et al., 2018). Therefore, we pose secondly that the availability of organic matter in the upper layers likely contributed to supplying remineralized P (organic P being generally more labile than other organic nutrients) (Vidal et al., 1999, 2003) and to enhancing the residence time of dFe originated from atmospheric deposition. These conditions all together would benefit the heterotrophic N2 fixers. Despite the fact that neither P* values from the BG2014/14 cruise (Table S1) nor climatological P* data for the Iberian Basin (World Ocean Atlas 2013 April to June average from 1955 to 2012, Figure 1 and Table S2) (Garcia et al., 2013) indicate a large PO43− excess (P* ranging between ~0.1 and 0.1 µmol L−1; Figure 1 and Tables S1 and S2). Spearman rank correlations suggest that volumetric N2 fixation rates were significantly correlated with the BG2014/14 shipboard P* values (p < 0.01). However, ENACWst stations Bel-11 and Bel-13 are weighing heavily in this correlation, and without the data from these two sites the correlation is no longer significant (p = 0.163). Landolfi et al. (2015) proposed that in waters depleted in DIN and PO43− and devoid of a positive P* signal, but replete in dFe, the ability of diazotrophs to draw N from the dissolved N2 pool confers them a competitive advantage over other phytoplankton for the use of dissolved organic phosphorus (DOP). According to Landolfi et al., the need for additional N and energy required for the enzymatic mineralization of DOP (synthesis of extracellular alkaline phosphatase) favors N2 fixers in such oligotrophic conditions, and such DOP utilization by diazotrophs has been reported in other studies for the North Atlantic (Dyhrman et al., 2006; Sohn and Capone, 2006; Moore et al., 2009). In case DOP had progressively accumulated towards the end of the spring bloom, it may have contributed to sustaining N2 fixation in the studied region. Atmospheric aerosol deposition determined during the GEOVIDE cruise (Shelley et al., 2017) as well as the satellite-based dust deposition values averaged over the month of May (Figure S3; Giovanni online satellite data system, NASA Goddard Earth Sciences Data and Information Services Center), both reveal weak dust loadings over the Iberian region at the time of sampling. While euphotic layer-integrated N2 fixation rates determined during both GEOVIDE and BG2014/14 cruises were negatively correlated with average May dust input (p < 0.01, Table S3), they tended to be positively correlated with the average dust input during the month preceding the field work (April). This correlation, though weak (p = 0.45, Table S3), suggests an availability of dFe in May likely resulting from the intense atmospheric dust input in the preceding month which affected the area studied here as well as the area more to the south. Indeed, it has been reported that dFe in surface waters (50 to 100 m deep) of the North Atlantic may remain available to the microbiota up to a month after the atmospheric dust deposition events, most likely due to the formation of ligands with dissolved organic components (Jickells, 1999; de Baar and de Jong, 2001; Sarthou et al., 2003). In addition it is likely that dFe was also supplied at the time of our sampling through lateral advection from the continental margin (for stations Geo-1 and Geo-2) as indicated by the surface salinity minima (Figures 2b and 2c) and the low dFe to dissolved aluminium ratios found there relative to surrounding waters (Tonnard et al., 2018). Also, dFe could have been supplied from the adjacent subtropical region more heavily impacted by the April dust deposition (stations Bel-11, Bel-13 and Geo-13). In addition, it is important to note that vertical mixing (related to post-winter convection) with Fe-enriched subsurface waters (Thuróczy et al., 2010; Rijkenberg et al., 2012; Garcia-Ibáñez et al., 2015) could also have supplied dFe particularly at station Geo-21 (to a lesser extent at Geo-13) where the surface waters showed rather nutrient replete conditions (Figure 3).

θ/S diagrams at stations Bel-11 and Bel-13 (and to a lesser extent at Geo-13) reveal the presence of very warm and saline waters at the surface and which appear to be advected from subtropical regions in the south, as indicated by SST satellite
images (Figure S2). Spearman rank correlations (Table S4) confirm that N₂ fixation rates increased towards elevated seawater temperature (\(p < 0.001\)) and NO\(_3^- + NO_2^-\) depleted surface waters (\(p < 0.05\)). We thus propose that N₂ fixation activity at stations Bel-11 and Bel-13, where rates were the highest, was stimulated by the northward advection of subtropical surface waters which would have received a larger supply of dFe from dust deposition (Figure S3A) and which carried positive P* signatures. Alternatively, these northward flowing waters could have conveyed active diazotrophs of subtropical origin. Shiozaki et al. (2013) reported a similar feature for the western North Pacific, where diazotrophic cyanobacteria were carried along warm surface currents while conserving their N₂ fixation potential. In contrast, in the central Bay of Biscay N₂ fixation was below detection limit at stations Bel-3 and Bel-5 (45.3–46.5° N) while it was still significant further offshore at similar latitude (station Geo-21, 46.5° N; Figure 7). During April 2014, dust deposition was lowest in the region of the Bay of Biscay (Figure S3), suggesting that N₂ fixation there might have been limited by dFe availability. This was corroborated by the observation that surface waters from stations Bel-3 and Bel-5, when incubated after dFe amendments, exhibited high N₂ fixation rates (> 25 nmol N L\(^{-1}\) d\(^{-1}\); Li et al., 2018).

The statements discussed in this section are further supported by the outcome of a multivariate statistical analysis providing a comprehensive view of the environmental features influencing N₂ fixation. A principal component analysis (PCA; Tables 2 and S2) generated two components (or axes) explaining 68% of the system’s variability. Axis 1 illustrates the productivity of the system, or more precisely the oligotrophic state towards which it is evolving. In fact, axis 1 is defined by a strong positive relation with surface temperature (reflecting the onset of stratification, particularly for stations Bel-11 and Bel-13; Figure 9) and an inverse relation with PP associated variables (PP, Chl \(_a\), NH\(_4^+\), NO\(_3^- + NO_2^-\)). Sites characterized by a moderate (Bel-3 and Bel-5) to high (Bel-7, Geo-21 and to a lesser extent Geo-13) primary production appear indeed tightly linked to these PP associated variables as illustrated in Figure 9. As reflected by the positive relation with surface salinity and P* (Figure 9), axis 2 denotes advection of surface waters of subtropical origin, for stations Bel-11, Bel-13 and Geo-13. For stations Geo-1 and Geo-2, the inverse relation with surface salinity (Figure 9) is interpreted to reflect fluvial inputs (Tonnard et al., 2018). Finally, this statistical analysis indicates that N₂ fixation activity was likely influenced by the two PCA components, tentatively identified as productivity (axis 1) and surface water advection (axis 2) from the shelf and the subtropical region.

This investigation into possible drivers of heterotrophic N₂ fixation in the Iberian Margin and Bay of Biscay points to the critical roles played by (i) organic matter availability in these open waters, resulting from the prevailing vernal bloom to post-bloom conditions, in combination with (ii) atmospheric dust deposition providing essential dFe. Further studies are required to investigate this possible link between heterotrophic N₂ fixation activity and phytoplankton bloom in the studied region. It is likely that surface water advection also played an important role in supporting N₂ fixation activities by conveying essential nutrients from subtropical regions or shelf areas into the studied region.

5 Conclusion

The present study highlights the occurrence of high N₂ fixation activity (81–1533 µmol N m\(^{-2}\) d\(^{-1}\)) in the temperate eastern North Atlantic off the Iberian Peninsula, under vernal bloom to post bloom conditions. However, no activity was detected in the central Bay of Biscay at the time of this study. We report diazotrophic activities being of similar range to ten times larger compared to those reported by others for the eastern tropical and subtropical North Atlantic. The qualitative assessments of \textit{nifH} diversity in the West Iberian Margin (from BG2014/14 and GEOVIDE cruises) suggest...
that the diazotrophic community was dominated by heterotrophs, among which the UCYN-A1 obligate symbiont of
photo-autotrophic prymnesiophyte cells as well as anaerobic bacteria being particle-associated or zooplankton symbionts,
such as Bacteroidetes, Proteobacteria and Firmicutes phylotypes. We postulate that the availability of suspended organic
matter (dissolved and/or particulate) related to the ongoing or past phytoplankton bloom promoted heterotrophic N₂
fixation activity by sustaining bacterial production, but also by providing sufficient nutrients essential for the nitrogenase
activity. Dissolved Fe was supplied mostly through atmospheric dust deposition, and advection from subtropical regions
or from the shelf area. The proposed environmental controls support the idea of a closer link between primary production
and N₂ fixation in productive areas where accumulation of organic matter would favour the growth of heterotrophic
diazotrophs. Further investigations of N₂ fixation activity, organic matter availability and assimilation off the Iberian
Margin particularly during productive seasons, are needed to confirm these statements.

Data availability. The data associated with the paper are available from the corresponding author upon request.

The Supplement related to this article is available.

Competing interests. The authors declare that they have no conflict of interest.

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**Table 1: Relative contribution (%) of N$_2$ fixation to Primary Production (PP).**

<table>
<thead>
<tr>
<th>Province</th>
<th>Station</th>
<th>Latitude (° N)</th>
<th>N$_2$ fixation contribution to PP (%) (Redfield 6.6 ratio)</th>
<th>SD</th>
<th>N$_2$ fixation contribution to PP (%) (mean POC/PN ratio of 6.3 ± 1.1)</th>
<th>SD</th>
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<tbody>
<tr>
<td>ENACWsp</td>
<td>Bel-3</td>
<td>46.5</td>
<td>0</td>
<td>-</td>
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<td></td>
<td>Bel-5</td>
<td>45.3</td>
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<td></td>
<td>Bel-7</td>
<td>44.6</td>
<td>0.4</td>
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<td>1</td>
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<td></td>
<td>Geo-21</td>
<td>46.5</td>
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<td>ENACWst</td>
<td>Bel-9</td>
<td>42.4</td>
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<tr>
<td></td>
<td>Bel-11</td>
<td>40.7</td>
<td>28</td>
<td>1.9</td>
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<td>1.8</td>
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<td></td>
<td>Bel-13</td>
<td>38.8</td>
<td>25</td>
<td>1.3</td>
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<td></td>
<td>Geo-1</td>
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<td>Geo-2</td>
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<td></td>
<td>Geo-13</td>
<td>41.4</td>
<td>3</td>
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<td>3</td>
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Table 2: Principal component matrix illustrating the components (or axis) loadings, in other words the correlation of each variable to a determined axis as obtained with the XLSTAT software. The percentage of variability of the system explained by each of the two axes is indicated, for a total explained variance of 68%.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Axis 1</th>
<th>Axis 2</th>
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<tr>
<td>Euphotic layer integrated primary production</td>
<td>-0.812</td>
<td>0.088</td>
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<tr>
<td>Euphotic layer averaged temperature</td>
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<td>0.130</td>
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<tr>
<td>Euphotic layer integrated Chl a</td>
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<td>Euphotic layer integrated [NH$_4^+$]</td>
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<td>-0.007</td>
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<tr>
<td>Euphotic layer integrated [NO$_3^-$ + NO$_2^-$]</td>
<td>-0.783</td>
<td>0.154</td>
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<tr>
<td>Climatological surface P* (20 m)</td>
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<tr>
<td>Euphotic layer averaged salinity</td>
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<tr>
<td>Dry + wet dust deposition (April 2014)</td>
<td>0.583</td>
<td>-0.423</td>
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<tr>
<td><strong>Euphotic layer integrated N$_2$ fixation</strong></td>
<td><strong>0.506</strong></td>
<td><strong>0.602</strong></td>
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Figure legends

**Figure 1:** Location of sampling stations during the Belgica BG2014/14 (black labels) and GEOVIDE (white labels) cruises (May 2014) superimposed on a map of the phosphate excess \((P^* = [PO_4^{3-}] – [NO_3^-] / 16)\) at 20 m depth seasonal average (April to June from 1955 to 2012; World Ocean Atlas 2013) (Garcia et al., 2013). Areas of dominance of the Eastern North Atlantic Central Waters of subpolar (ENACWsp) and subtropical (ENACWst) origins are separated by a blue dashed line. Black dashed and solid contour lines illustrate 500 m and 1500 m isobaths, respectively.

**Figure 2:** O/S diagrams obtained using CTD profiles from surface layer down to the 1500 m depth during (a) the Belgica BG2014/14 cruise (stations Bel-3, 5, 7, 11 and 13), (b) the GEOVIDE cruise (stations Geo-1, 2, 13 and 21) and (c) both expeditions combined. Diamonds indicate the characteristics of the major water masses encountered as presented in Fiúza (1984) and García-Ibáñez et al. (2015): Eastern North Atlantic Central Waters (ENACW) of subpolar (ENACWsp) and subtropical (ENACWst) origins, Mediterranean Water (MW) and Labrador Sea Water (LSW).

**Figure 3:** Spatial distribution of Chl a (a, d), NH\(_4\)\(^+\) (b, e) and NO\(_3^-\) + NO\(_2^-\) (c, f) concentrations along the Belgica BG2014/14 (a to c) and GEOVIDE (d to f) transects. Sampling stations, and the area of dominance of Eastern North Atlantic Waters of subpolar (ENACWsp) and subtropical (ENACWst) are illustrated according to the latitudinal and longitudinal range of each transect. Mixed layer depths (MLD, black lines connecting diamonds) was estimated using a temperature threshold criterion of 0.2°C relative to the temperature at 10 m (de Boyer Montégut et al., 2004).

**Figure 4:** Spatial distribution (± SD) of depth-integrated primary production (duplicates in light and dark green; mmol C m\(^{-2}\) d\(^{-1}\)) determined during (a) the Belgica BG2014/14 and (b) GEOVIDE cruises. Error bars represent the propagated measurement uncertainty of all parameters used to compute volumetric uptake rates.

**Figure 5:** Time series of area-averaged chlorophyll a concentration (mg m\(^{-3}\)) over the period between December 2013 and December 2014 for the 0.5° x 0.5° grid surrounding each sampled station during (a) the Belgica BG2014/14 and (b) GEOVIDE cruises, registered by Aqua Modis satellite (Giovanni online satellite data system). Dashed box illustrated the sampling period for both cruises (May 2014).

**Figure 6:** Relative importance of euphotic layer integrated taxa-specific pigments at the four sites sampled during the GEOVIDE cruise according to Tonnard et al. (in preparation for this Special Issue).

**Figure 7:** Spatial distribution (± SD) of depth-integrated N\(_2\) fixation rates (duplicates in light and dark blue; µmol N m\(^{-2}\) d\(^{-1}\)) determined during (a) the Belgica BG2014/14 and (b) GEOVIDE cruises. Error bars represent the propagated measurement uncertainty of all parameters used to compute volumetric uptake rates.

**Figure 8:** Diversity of nifH sequences recovered during the Belgica BG2014/14 cruise; only detectable at stations Bel-11 and Bel-13. The number of sequences per group is indicated inside the bars for a total of 103 sequences recovered (a). The clone-based rarefaction curve (b), was produced by repeatedly re-sampling randomly among all clones and plotting the average number of OTU represented at each step (from 1 to the maximum number of clones, 41 and 62, for stations Bel-11 and Bel-13, respectively). The flattening of the curve indicates that a reasonable number of clones have been recovered and that only the rarest OTUs remained unsampled (Gotelli and Colwell, 2001).
Figure 9: Euclidean distance biplot illustrating the axis loadings corresponding to the two components as obtained from the result of PCA based on Spearman rank correlation with depth-integrated rates of N$_2$ fixation and primary production (PP), phosphate excess (average P* at 20 m depth surrounding each sampled site from the April to June; World Ocean Atlas 2013 climatology between 1955 to 2012) (Garcia et al., 2013), average dust dry + wet deposition derived during April 2014 satellite data (Giovanni online data system, NASA Goddard Earth Sciences Data and Information Services Center) and ambient variables (temperature, salinity, and nutrient data). Coloured dots represent the projection of each station corresponding to their biogeochemical characteristics.
Figure 1
Belgica BG2014/14

GEOVIDE

Figure 3
Figure 4
Figure 5

(a) and (b) show the changes in Chl a (mg m⁻³) over time from December 2013 to December 2014. The graphs illustrate the seasonal variation in chlorophyll levels, with peaks in April and May.
Figure 6

Pigment type

- Chl b
- Zeaxanthin
- Peridinin
- Fucoxanthin
- Alloxanthin
- 19'-Butanoyloxyfucoxanthin
- 19'-Hexanoyloxyfucoxanthin

Taxonomic significance

- Green algae & Prochlorophytes
- Cyanobacteria & Prochlorophytes
- Dinoflagellates
- Diatoms
- Cryptophytes
- Pelagophytes
- Prymnesiophytes
Figure 7
Figure 8
Figure 9

Biplot (axes F1 and F2: 68.45%)

Active variables • Active observations