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# Planktonic dinitrogen fixation along a longitudinal gradient across the Mediterranean Sea during the stratified period (BOUM cruise)

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**Abstract.** This study provides extensive data on planktonic  $N_2$  fixation rates across the whole Mediterranean Sea. They show that  $N_2$  fixation occurs in Mediterranean waters during the stratification period, with a clear decreasing trend from the oligotrophic western basin ( $10\text{--}76\ \mu\text{mol m}^{-2}\text{ d}^{-1}$ ) to the ultra oligotrophic eastern basin ( $0\text{--}0.4\ \mu\text{mol m}^{-2}\text{ d}^{-1}$ ). Highest rates are measured in the less oligotrophic western basin, between the surface and 75 m-depth, where 45 to 75 % of  $N_2$  fixation are found within the picoplanktonic fraction ( $<3\ \mu\text{m}$ ). While the biogeochemical impact of  $N_2$  fixation in the eastern basin seems negligible,  $N_2$  fixation is able to sustain up to 35 % of new primary production during the stratified period in the western basin. These data disagree with indirect estimates of  $N_2$  fixation based on geochemical tracers and nutrient budgets, which indicates that  $N_2$  fixation increases with increasing N:P ratios and decreasing stable N isotopic signature of particulate organic nitrogen and  $\text{NO}_3^-$  from west to east. These results finally point out the need to assess  $N_2$  fixation at a higher temporal resolution in order to better understand the diazotrophs' dynamic under contrasted biogeochemical conditions.

## 1 Introduction

The concept of Redfield ratios (1934) has been fundamental to our understanding of the biogeochemistry of the oceans. Redfield (1934) proposed that the N:P ratio of plankton (16:1) causes the ocean to have a remarkably similar ratio

of dissolved nitrate ( $\text{NO}_3^-$ ) and phosphate ( $\text{PO}_4^{3-}$ ). While this canonical value proposed by Redfield is still a reference, several studies conducted in the ocean over the past decades show anomalies in the ratios; for example non-Redfieldian elevated ratios of  $\text{NO}_3^-$  to  $\text{PO}_4^{3-}$  produced from regenerated particulate material are indicative of  $N_2$  fixation inputs (e.g. Gruber and Sarmiento, 1997).

The Mediterranean Sea is an interesting oceanic environment in which nutrient ratios diverge greatly from canonical Redfieldian values. Intermediate and deep waters display gradually increasing N:P ratios from 20:24:1 in the western basin to 28:1 in the eastern basin (Mc Gill, 1965, 1969; Coste and Minas, 1967; Bethoux et al., 1998; Krom et al., 1991; Moutin and Raimbault, 2002; Pujo-Pay et al., 2011). While several “conflicting” hypotheses have been proposed over the last twenty years to explain this anomaly, it still represents an open issue for the oceanographic community. In the first hypothesis, based on nutrient budgets for the Mediterranean Sea, the anomalous N:P ratio is explained by the excess of nitrogen relative to phosphate in all nutrient sources arriving to the basin, associated to low denitrification rates (Krom et al., 2004). In particular, the Mediterranean Sea receives among the highest rates of aeolian material deposition in the global ocean (Guerzoni et al., 1999), which provide dissolved nitrogen and phosphorus in a molar ratio increasing from 60:1 in the western basin to 105:1 in the eastern basin (Markaki et al., 2010). In addition, the eastern Mediterranean Sea receives seawater inflow from the Black Sea and major rivers such as the Nile and the Po, which also provide nutrients in a ratio greatly in excess to 16:1 (Krom et al., 2004).

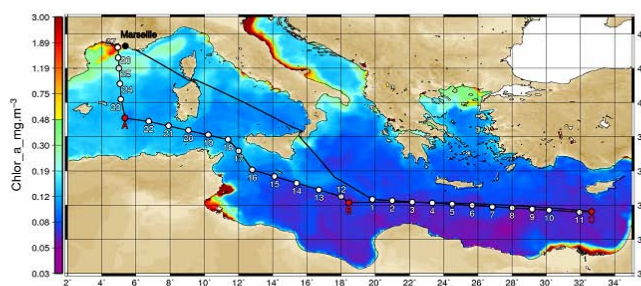
An alternative explanation proposes that elevated N:P ratios are due to intense dinitrogen ( $N_2$ ) fixation, the prokaryotic-mediated conversion of atmospheric dinitrogen



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into the bioavailable form of ammonia. This process provides a source of N, not associated with a concomitant input of P, thus increasing seawater N:P ratios. Indirect evidences based on nutrient budgets (Bethoux and Copin-Montegut, 1986) first proposed that  $N_2$  fixation could be the source of the observed excess nitrate to the Mediterranean. Later, Sachs and Repeta (1999) and Pantoja et al. (2002) reinforced this hypothesis through stable N isotope studies and provided evidence for significant  $N_2$  fixation in the present and recent geological times. Pantoja et al. (2002) revealed an eastward decrease in surface  $^{15}N$  (‰) of suspended particulate organic nitrogen (PON) ( $2.7 \pm 1.2$  ‰ to  $-0.2 \pm 0.7$  ‰), chlorophyll-*a* ( $2.6 \pm 2.3$  ‰ to  $-7.1 \pm 1.3$  ‰) and deep-water nitrate ( $3.4 \pm 0.5$  ‰ to  $2.5 \pm 0.1$  ‰), implying an eastward increase in the contribution of  $N_2$  fixation to the water column N budget. They estimated that  $N_2$  fixation accounted for 20 to 90 % of the N supply to the western and eastern Mediterranean, respectively, exceeding previous estimates (7–40 %) based on nutrient budgets (Bethoux and Copin-Montegut, 1986). Besides these indirect geochemical estimates, few direct measurements have yet been reported for the Mediterranean Sea. Concerning  $N_2$ -fixing organisms, some free trichomes of *Trichodesmium* spp. have been detected in the 60's (Margalef, 1969), but large blooms of *Trichodesmium* spp. have never been observed in the Mediterranean Sea, probably because phosphate availability never reaches the critical level allowing them to grow (Moutin et al. 2005, Moutin et al. 2008). However, molecular studies conducted over coastal stations in the eastern and western basin have clearly reported the presence of diazotrophic microorganisms related to Archaea, Proteobacteria and/or Cyanobacteria (Man-Aharonovich et al., 2007; Le Moal and Biegala, 2009), and of the diatom symbiont *Richelia intracellularis* (Bar Zeev et al., 2008). The potential of these organisms to bloom and fix dinitrogen at high rates in the P-depleted Mediterranean waters is still in debate; recent studies have indeed reported during the summer stratification period either high  $N_2$  fixation rates at one isolated station in the Levantine basin (Rees et al., 2006), or extremely low rates at six stations distributed across the basin (Ibello et al., 2010). This leads to a difficulty in concluding about the biogeochemical importance of diazotrophy in this environment.

During the BOUM operation (Biogeochemistry from the Oligotrophic to the Ultra-oligotrophic Mediterranean, June–July 2008), we had the opportunity to perform  $N_2$  fixation measurements over a large number of stations (17) along a 2000 km transect in the Mediterranean Sea (Fig. 1), exhibiting nutrient and chlorophyll gradients. The objectives of this study were (i) to quantify  $N_2$  fixation rates along the transect during the stratification period, (ii) to determine the relative importance of the large ( $>3 \mu m$ ) and the small ( $<3 \mu m$ ) size fractions to the bulk nitrogen fixation, (iii) to estimate the contribution of fixed dinitrogen to the nitrogen budget of the Mediterranean Sea during the stratification period.



**Fig. 1.** Transect of the BOUM cruise superimposed on a SeaWiFS surface Chl-*a* composite image (June 2008), and location of the fourteen short (numbers) and three LD stations (letters) of the cruise sampled for  $N_2$  fixation during the cruise.

## 2 Material and methods

This research was carried out in the Mediterranean Sea on-board the R/V *Atalante* in the framework of the BOUM project. The transect started in La Seyne sur Mer, France ( $43^{\circ}07'N$ ,  $05^{\circ}52'E$ ) on 16 June 2008, stretched to the eastern basin ( $42^{\circ}50'N$ ,  $38^{\circ}50'E$ ) and ended in La Seyne sur Mer ( $43^{\circ}07'N$ ,  $05^{\circ}52'E$ ) on 20 July 2008 (Fig. 1). The Mediterranean Sea has long been recognized as a low nutrient concentration basin (Mc Gill, 1965; Krom et al., 1991), exhibiting increasing oligotrophy from west to east. It is characterized by seasonal variability in hydrological structure and trophic regimes, ranging from a strong thermal stratification with a sharp thermocline (10–20 m deep) during summer and fall, associated with an efficient pycnocline acting as a physical barrier. During the mixing period in winter, nutrients are brought to the surface layer and can allow phytoplankton to bloom in the early spring.

### 2.1 Nitrogen fixation measurements

Rates of nitrogen fixation were measured using the  $^{15}N_2$  tracer method (Montoya et al., 1996). Water samples were collected using 12 L Niskin bottles mounted on a seabird CTD rosette sampler (model SBE 911) at seventeen stations (Fig. 1), including fourteen short duration (4 h) stations (SD stations #1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 24, 25, 27) and three long duration (4 days) process study stations (LD stations #A, B, C, respectively located in the Algero-Provencal, the Tyrrhenian, and the Levantine basins). LD stations were located in the center of anticyclonic gyres, where the lateral advection was expected to be low.

For each LD station, nitrogen fixation rates were measured at nine depths (75 %, 50 %, 35 %, 20 %, 10 %, 3 %, 1 %, 0.3 % and 0.1 % surface irradiance levels, corresponding to sub-surface down to 130, 160 and 145 m for stations #A, B, C, respectively). The same profile was repeated twice at each station. For each SD station, they were performed only at two depths (50 % and 3 % surface

light levels, corresponding to the subsurface and the upper part of the deep chlorophyll maximum (DCM+)). One extra depth was sampled at stations #15, 19 and 24 at mid-depth between surface and DCM+. All CTD profiles are available on the BOUM website (<http://www.com.univ-mrs.fr/BOUM/spip.php?rubrique6>). Nitrogen fixation rates measurements were performed in acid-washed (10 % dilution of 32 % HCl) 4.5 L polycarbonate bottles equipped with septum caps to which additions of 4 mL of  $^{15}\text{N}_2$  gas (99 %, EURISOTOP) were made using a gas-tight syringe. Each bottle was then shaken 10 times. Incubations were always started before dawn and lasted 24 h. At LD stations, incubations were performed in situ on a drifting mooring line situated at the same depth from which the samples were collected. At SD stations, incubations were performed in on-deck incubators equipped with circulating seawater at the specified irradiances using blue screening. In both cases, incubations were terminated by gently filtering samples; at SD stations and LD stations at day 1, for each depth, samples were filtered under low vacuum pressure ( $<100$  mm Hg) onto pre-combusted (4 h at  $450^\circ\text{C}$ ) GF/F filters (25 mm diameter,  $0.7\ \mu\text{m}$  nominal porosity) for determination of the “bulk” nitrogen fixation. At LD stations at day 3, for each depth, one replicate was filtered following the same procedure and one more replicate per depth was size-fractionated: it was pre-filtered onto  $3\ \mu\text{m}$  polycarbonate filters for fraction  $>3\ \mu\text{m}$ , while the filtrate was collected onto a pre-combusted GF/F filter for analysis of the  $<3\ \mu\text{m}$  fraction. Sample filters were stored in 2 ml glass tubes and dried at  $60^\circ\text{C}$ , then stored over desiccant until analysis.

## 2.2 Mass spectrometry analysis

The isotopic enrichment analysis was performed by continuous-flow isotope ratio mass spectrometry using an Integra-CN mass spectrometer according to Montoya et al. (1996). The accuracy of the system was verified regularly using reference material (IAEA, International Atomic Energy Agency, Analytical Quality Control Services). The isotopic enrichment was calibrated using IAEA reference material every 10 samples. Before samples analysis, we verified the linearity of  $^{15}\text{N}$  atom % as a function of increasing particulate nitrogen mass on both natural and  $^{15}\text{N}$  enriched material, and the unchanging response of  $^{15}\text{N}$  atom % within the range of particulate nitrogen in our samples. This step is critical in ultra-oligotrophic environments such as the Mediterranean Sea or subtropical gyres.  $^{15}\text{N}$  atom % was linear (Fisher test,  $p < 0.01$ ) between 0.20 and  $39\ \mu\text{mol N}$ , which is within the range of particulate nitrogen measured in all our samples (0.45 to  $3.65\ \mu\text{moles N}$ ). Detection and quantification limits for particulate nitrogen were calculated daily as 3 times and 10 times the standard deviation on 10 blanks analysis, respectively. Detection limits ranged 0.08 to  $0.15\ \mu\text{moles}$ , and quantification limits ranged 0.10 to  $0.19\ \mu\text{moles N}$ . The  $^{15}\text{N}$  isotope enrichment of a sample was

calculated using the  $^{15}\text{N}$  atom % excess over time, over the  $^{15}\text{N}$  atom % in samples taken from the same station at time zero (natural abundance of  $^{15}\text{N}$ ). The value of time zero enrichment was determined on bottles filtered immediately after adding  $^{15}\text{N}_2$ . We considered the results to be significant when  $^{15}\text{N}$  excess enrichments were greater than three times the standard deviation obtained with eight time zero samples.

For every measurement presented in the result section, uncertainties were calculated using partial derivation as propagation of uncertainties. The expanded measurement uncertainty is used, with a coverage factor  $k = 2$  (i.e. confidence interval of 95 %).

## 2.3 Temperature and chlorophyll measurements

Temperature profiles were obtained using a temperature sensor on the CTD. The sensor was calibrated from the drift before and after the cruise. Chlorophyll a concentrations have been derived from fluorescence data measured on CTD profiles using a SeaTech fluorimeter. Fluorescence data have been converted into chlorophyll concentrations using the calibration performed during the cruise using the true chlorophyll a concentrations measured by HPLC (Crombet et al., 2011) following the equation:

$$[\text{Chl} - a] = 3.006302 \times \text{Fluorescence Unit} - 0.00722932.$$

## 3 Results

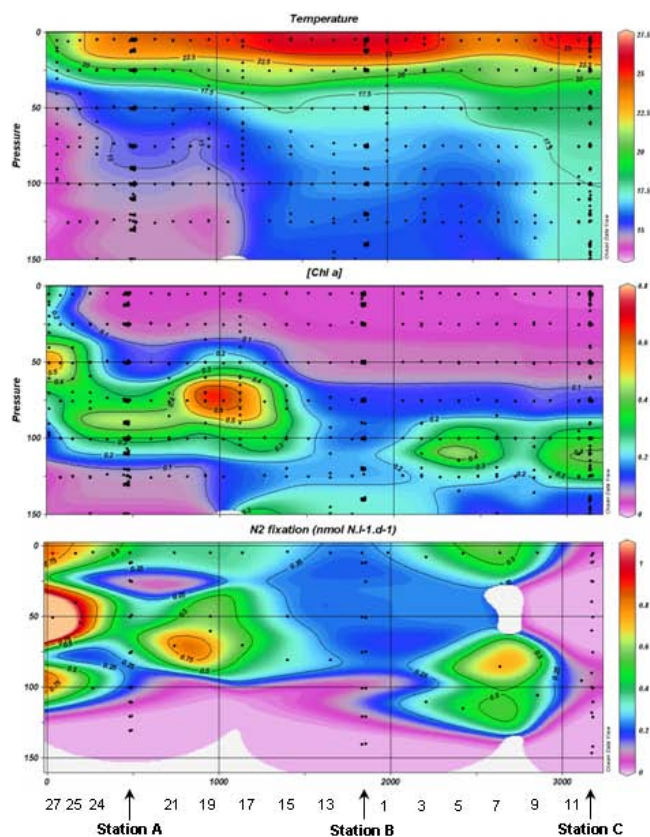
### 3.1 Biogeochemical context

Surface temperature ranged from  $22\text{--}24^\circ\text{C}$  in the western basin to  $22\text{--}27^\circ\text{C}$  in the eastern basin (Fig. 2). The water column was characterized by a warm surface mixed layer of 7.8, 7.2, 10.1 m depth at LD stations A, B and C, respectively (Fig. 3).

Chlorophyll concentrations (Fig. 2) ranged from 0.03 to  $0.07\ \mu\text{g l}^{-1}$  the surface mixed layer, except in the Rhone river plume, where concentrations reached up to  $0.4\ \mu\text{g l}^{-1}$ . The whole section is characterized by a clear deepening of the Deep Chlorophyll Maximum from west (40–80 m) to east (80–125 m), associated with the increasing trend in oligotrophy.

Nutrient data indicate that surface waters were depleted in nutrients (Pujo-Pay et al., 2011): concentrations of nitrate + nitrite and phosphate were low and close or below the detection limit of conventional micromolar methods (0.02 and  $0.01\ \mu\text{M}$  for nitrate and phosphate respectively). The thickness of this depleted layer increased towards the East from about 10 m in the Gulf of Lion to more than 100 m in the Levantine basin.





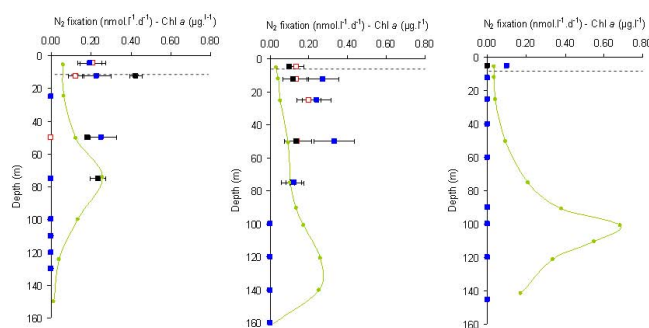
**Fig. 2.** Longitudinal cross section of  $N_2$  fixation rates ( $\text{nmol l}^{-1} \text{d}^{-1}$ ) along the BOUM transect (0–160 m) including fourteen SD stations and the three LD stations. X axis: distance in km from the station 27.

### 3.2 Nitrogen fixation rates: “bulk” versus “size fractionation”

The overall range of  $N_2$  fixation rates measured over the entire Mediterranean transect was  $0.10\text{--}1.80 \text{ nmol l}^{-1} \text{d}^{-1}$ . They exhibited strong longitudinal gradients (Fig. 2), the lowest rates being measured in the eastern basin, averaging  $0.43 \pm 0.33 \text{ nmol l}^{-1} \text{d}^{-1}$ . Rates increased toward the western basin (average:  $0.63 \pm 0.45 \text{ nmol l}^{-1} \text{d}^{-1}$ ) to reach maximum values in the Rhone river plume, with rates reaching  $1.80 \pm 0.19 \text{ nmol l}^{-1} \text{d}^{-1}$  at 40 m-depth at station #27. This increasing longitudinal gradient is confirmed by vertical profiles obtained at LD stations A, B and C (Fig. 3), exhibiting extremely low rates at the most eastern station C compared to stations B ( $0.10\text{--}0.33 \text{ nmol l}^{-1} \text{d}^{-1}$ ) and A ( $0.19\text{--}0.43 \text{ nmol l}^{-1} \text{d}^{-1}$ ) located in the center and the western Mediterranean Sea, respectively.

Data indicate that rates were lower at the three LD stations A, B, and C (located in the center of anticyclonic gyres), compared to the ones measured at SD stations.

Data from the three vertical profiles obtained at LD stations A, B and C (Fig. 3) indicate that rates were not uniform



**Fig. 3.** Vertical profiles of  $N_2$  fixation fluxes ( $\text{nmol l}^{-1} \text{d}^{-1}$ ) measured at LD stations A (a), B (b) and C (c). Black squares: bulk  $N_2$  fixation measured at day 1. Light blue squares: bulk  $N_2$  fixation measured at day 3 at the same station. Red squares:  $N_2$  fixation in the picoplanktonic ( $<3 \mu\text{m}$ ) fraction at day 3. Error bars represent uncertainties on the measurement. Green circle: Chl-*a* concentrations ( $\mu\text{g l}^{-1}$ ). Dashed line represent the mixed layer depth.

throughout the water column. At stations A and B,  $N_2$  fixation was measurable between the surface and 75 m-depth, and became undetectable from 100 to 160 m-depth. Data obtained at SD stations (only surface and DCM+ depths were sampled) indicate that rates were globally higher at depth (DCM+) compared to those measured in surface at most stations. For the three SD stations (#15, 19, 24) for which an extra intermediate depth between surface and DCM+ was sampled, data indicate that they exhibit the maximum rates at this intermediate depth (around 50 m-depth) within the euphotic zone.

Depth integrated rates were calculated at stations #A, B, C, 15, 19, 24 (Table 1) and show a decrease from the western basin ( $10.2 \pm 2.7$  to  $76.2 \pm 7.7 \mu\text{mol l}^{-1} \text{d}^{-1}$  at station B and 24, respectively, Table 1) to the eastern basin ( $0.4 \pm 0.1 \mu\text{mol l}^{-1} \text{d}^{-1}$  at station C). Size fractionation experiments performed at LD stations (Fig. 3) indicate that a significant part of the nitrogen fixation rates was associated with the picoplanktonic size fraction (fraction  $<3 \mu\text{m}$ ), which accounted for 45 and 75 % of total nitrogen fixation at station A and B, respectively.  $N_2$  fixation rates in this picoplanktonic fraction followed the same vertical pattern at station B, where the maximum rates were located between the surface and 50 m-depth. At station A, maximum rates associated with this fraction were reached at the surface (5–10 m).

## 4 Discussion

### 4.1 $N_2$ fixation rates, size fractionation and diazotrophic communities

This study provides one of the largest spatial datasets of  $N_2$  fixation rates to date available for the Mediterranean Sea, covering a large range of trophic conditions from the ultra-oligotrophic eastern basin to the less oligotrophic western

**Table 1.** Integrated primary production, N<sub>2</sub> fixation, vertical nitrate diffusion at the base of the euphotic zone and atmospheric deposition at stations A, B, C, 15, 19 and 24 and percentage of estimated “new” primary production (New PP) sustained by each source of “new” nitrogen. New PP (expressed in  $\mu\text{mol N m}^{-2} \text{d}^{-1}$ ) has been considered as 10 % of total PP (Moutin and Raimbault, 2002).

Location	Primary production* $\text{mmol C m}^{-2} \text{d}^{-1}$	N <sub>2</sub> Fixation $\mu\text{mol N m}^{-2} \text{d}^{-1}$	% New PP	Vertical nitrate diffusion $\mu\text{mol N m}^{-2} \text{d}^{-1}$	% New PP	N atmospheric deposition $\mu\text{mol N m}^{-2} \text{d}^{-1}$	% New PP
St A	13–13.7	$10.2 \pm 2.7/14.8 \pm 1.5$	6–9	9–13	5–8	16–34	9–20
St B	15.5–16.3	$11.8 \pm 5.6/18.6 \pm 4.6$	7–9	5–13	7–25	10–27	5–13
St C	11.4–20	$0.4 \pm 0.1/0 \pm 0$	0–0.3	2–6	0.1–4	10–27	4–18
St 15	16.7–33.3	$24.6 \pm 3.8$	6–11	16–23	4–11	60**	14–28
St 19	16.7–33.4	$35.9 \pm 4$	8–17	40–59	14–18	60**	15–28
St 24	16.7–33.5	$76.2 \pm 7.8$	17–35	30–45	7–21	60**	16–28

\* data from Christaki et al. (2011) for stations A, B, C and range given by measurements in the same area during the MINOS cruise (Moutin and Raimbault, 2002) for stations 15, 19, 24

\*\* data from Sandroni et al. (2007) for the western Mediterranean Sea at the same season

basin during the stratification period. The presented data report that N<sub>2</sub> fixation is an active process at that period along the transect, with rates ranging from  $0.10$ – $1.80 \text{ nmol l}^{-1} \text{d}^{-1}$ , the lowest being observed in the eastern basin and the highest in the western basin, close to the coast in the Rhone river plume. These results are in agreement with molecular data obtained by Le Moal et al. (2011), who used whole-cell hybridization of specific Nitro821 oligonucleotide probe to quantify unicellular diazotrophic cyanobacteria in three size fractions over the same transect. They report that unicellular diazotrophic cyanobacteria were recovered at all stations across the transect with higher cell concentrations in the western basin compared to the eastern one. These two studies thus confirm the presence of actively-fixing diazotrophs throughout the Mediterranean Sea during the stratification period.

Our data report that 45 to 75 % of N<sub>2</sub> fixation rates were recovered in the picoplanktonic size fraction ( $<3 \mu\text{m}$ ), which is in accordance with TSA-FISH data from Le Moal et al. (2011), showing that the community of unicellular diazotrophs was dominated by picoplanktonic diazotrophic cyanobacteria. 16S and *nifH* phylogenies revealed their affiliation to Group A, *Bradyrhizobium* and  $\alpha$  proteobacteria.

N<sub>2</sub> fixation rates measured in the size fraction  $>3 \mu\text{m}$  could also be due to picoplanktonic unicellular diazotrophic cyanobacteria, as Le Moal et al. (2011) have shown that 25 % of this community was recovered within the  $3$ – $10 \mu\text{m}$  and  $>10 \mu\text{m}$  size fractions, in association with unthecated dinoflagellates. Similar results were observed in the Equatorial Pacific Ocean (Bonnet et al., 2009), where up to 60 % of unicellular diazotrophic cyanobacteria were recovered in large size fractions, indicating that these unicellular forms may be responsible for some rates formally attributed to organisms  $>3 \mu\text{m}$  such as *Trichodesmium* spp. or *Richelia* spp. Nonetheless, phytoplankton net hauls revealed the presence of the filamentous cyanobacterium *Richelia intracellularis* at all stations sampled across the entire basin during the BOUM cruise (Crombet et al., 2011), in associa-

tion with the centric diatoms *Hemiaulus hauckii* and *Rhizosolenia styliformis*. Crombet et al. (2011) report from cell counts performed at discrete depths that the diatom *Hemiaulus hauckii* is the most abundant diatom at the deep silica maximum located close to the DCM. *Richelia* in association with *Hemiaulus* is thus suspected here (along with possible picoplanktonic unicellular diazotrophic cyanobacteria associated to nano- and micro-particles) to be responsible for the N<sub>2</sub> fixation rates measured at depth ( $50$ – $60 \text{ m}$ ), especially at stations #17 to 19 around  $80 \text{ m}$ -depth, where *Hemiaulus* reached a maximum of  $250 \text{ cells l}^{-1}$  (F. Gomez, personal communication, 2009), corresponding to N<sub>2</sub> fixation rates of  $0.51$  to  $0.72 \text{ nmol l}^{-1} \text{d}^{-1}$ , and at stations located in the Rhone river plume where they reach up to  $150 \text{ cells l}^{-1}$ , corresponding to N<sub>2</sub> fixation rates of  $1.06$  to  $1.80 \text{ nmol l}^{-1} \text{d}^{-1}$ . This type of association between *Richelia* and centric diatoms has already been observed in eastern Mediterranean waters off the coast of Israel (Bar Zeev et al., 2008). Finally, we cannot exclude the contribution of *Trichodesmium* spp. to the N<sub>2</sub> fixation rates measured in the  $>3 \mu\text{m}$  size fraction in this study, as few trichomes have been observed in phytoplankton net hauls but cannot be quantified with this sampling method (Crombet et al., 2011).

## 4.2 Biogeochemical significance and potential controlling factors of N<sub>2</sub> fixation in the Mediterranean Sea

### 4.2.1 Eastern Mediterranean Sea (Stations B to C)

This study indicates that N<sub>2</sub> fixation rates decreased when going eastward, indicating possible different biogeochemical/nutrient forcing on diazotrophs within the different basins. In the eastern basin, N<sub>2</sub> fixation rates were low, especially at station C where they reached  $0.4 \pm 0.1 \mu\text{mol m}^{-2} \text{d}^{-1}$ , which is in agreement with recent data obtained by Ibello et al. (2010) who also reported extremely low rates ( $0.5$  to  $2 \mu\text{mol m}^{-2} \text{d}^{-1}$ ) at three stations in the Levantine basin in May–June 2007. These low rates

**Table 2.** Examples of studies showing the range of oceanic N<sub>2</sub> fixation areal rates measured in some contrasting oceanic environments.

Location $\mu\text{mol N m}^{-2} \text{ d}^{-1}$	Areal rates	Source
Tropical North Pacific (ALOHA)	69	Dore et al. (2002)
Tropical Atlantic	86	Goering et al. (1966)
Tropical Atlantic	24	Voss et al. (2004)
Tropical Atlantic	59–898	Capone et al. (2005)
Eastern Tropical North Pacific	520	Montoya et al. (2004)
Equatorial Pacific	18–358	Bonnet et al. (2009)
Bermuda	41–93	Orcutt et al. (2001)
Arabian Sea	35–129	Capone et al. (1998)
North East Pacific (California)	0–15	Needoba et al. (2007)
Eastern Mediterranean Sea	0–0.4	This study
Western Mediterranean Sea	10–76	This study

during the stratification period are also confirmed by further measurements carried out at five stations in September 2008 during a SESAME cruise on a transect across the Levantine basin to Crete ( $0.9$  to  $3 \mu\text{mol m}^{-2} \text{ d}^{-1}$ , Yogeve et al., 2011), as well as by the annual N<sub>2</sub> fixation cycle performed by Bar Zeev et al. (2008). All these concordant studies report extremely low rates compared to measurements performed in the tropical and subtropical Atlantic and Pacific Oceans (Table 2), indicating a potential nutrient limitation for the extensive development of N<sub>2</sub>-fixing organisms during this season. At the time of the cruise, surface temperatures ( $24$ – $27^\circ\text{C}$ ) may not be limiting for N<sub>2</sub> fixers development (e.g. Breitbart et al., 2007; Webb et al., 2009). This decreasing trend from west to east is not in accordance with previous isotopic data (Pantoja et al., 2002) reporting an eastward increase in the contribution of N<sub>2</sub> fixation to the water column N budget. These authors concluded, using isotopic mass balance, that up to 90 % of nitrate uptake in the Eastern Mediterranean basin derives from biological N<sub>2</sub> fixation. On the basis of a C:N = 6.6, the contribution of N<sub>2</sub> fixation to the nitrogen demand of “new” primary production at station C (considered as 10 % of primary production presented in Christaki et al., 2011) is negligible (0–0.3 %, Table 1), indicating on the opposite a minor contribution of N<sub>2</sub> fixation at the studied period.

As a first approximation, anticyclonic eddies may be considered as closed systems. Then, in addition to N<sub>2</sub> fixation, the other sources of “new” nitrogen to the euphotic zone during intense summer stratification are vertical diffusive fluxes from below and atmospheric deposition. The vertical NO<sub>3</sub><sup>−</sup> flux in the euphotic layer was calculated from the product of the vertical eddy diffusivity coefficient ( $K_z$ , in  $\text{m}^2 \text{ s}^{-1}$ ) measured at every LD station using a SCAMP microprofiler (Cuyper et al., 2011), and the NO<sub>3</sub><sup>−</sup> gradient at the nitracline following calculations detailed in Moutin et al. (2011) (Table 3). The “new” N provided by atmospheric deposition was calculated based on aerosol mass collected at every LD station during the cruise (Ternon et al., 2011), to which

we applied a mean deposition velocity of particles of  $0.5$  to  $1 \text{ cm s}^{-1}$  (Duce et al., 1991; Sandroni et al., 2000) and a total N content based on data from Markaki et al. (2003) for the eastern basin and from Loye-Pilot et al. (1990) for the western basin. Those calculations performed at station C resulted in diffusive nitrate fluxes of  $2$ – $6 \mu\text{mol m}^{-2} \text{ d}^{-1}$ , and total atmospheric dissolved nitrogen deposition of  $10$ – $27 \mu\text{mol m}^{-2} \text{ d}^{-1}$  (Table 1), indicating that atmospheric deposition of nitrogen fuels most of new primary production at this station during intense summer stratification, and the contributions of N<sub>2</sub> fixation and vertical nitrate diffusion in the euphotic zone are negligible. Further studies would be needed to precise the atmospheric numbers, mainly by performing direct N dissolution experiments. However, they are in the range reported for the eastern Mediterranean Sea (Markaki et al., 2010). The diffusive NO<sub>3</sub><sup>−</sup> fluxes are at the lower range of those estimated by Moutin and Raimbault (2002) across the Levantine basin, but are probably more accurate because based on direct measurements of eddy diffusion coefficient ( $K_z$ ) during the cruise, while Moutin and Raimbault (2002) estimated the  $K_z$  from the turbulent kinetic energy dissipation rate ( $\varepsilon$ ) and the buoyancy frequency  $N(z)$  according to Osborn (1980). However, those direct measurements of  $\varepsilon$  and  $K_z$  may be refined in the future using a larger number of SCAMP profiles to catch the variability associated with turbulence. All these external sources of N together sustain only <10 % of total primary production at station C, indicating that regeneration of nutrients through the food web of surface waters mainly sustains primary production at station C, as previously observed by Moutin et Raimbault (2002) in the whole eastern basin.

At the scale of the Eastern Mediterranean Sea, if we assume that N<sub>2</sub> fixation occurs 6 months per year (approximately the length of the strongest stratification period) with mean areal rates of  $0.4$ – $3.0 \mu\text{mol N m}^{-2} \text{ d}^{-1}$  (This study, Ibello et al., 2010; Yogeve et al., 2011), the total N supply provided by N<sub>2</sub> fixation to the eastern Mediterranean Sea is  $0.1$ – $0.7 \times 10^9 \text{ N yr}^{-1}$  (Table 4), which accounts for only 0.1–0.4 % of the total external annual N inputs (excluding exchanges at the straits and winter convection) over the eastern basin ( $160 \times 10^9 \text{ mol N yr}^{-1}$ ), which are mainly driven by atmospheric deposition and river discharge (Table 4; Krom et al., 2010). This result is two orders of magnitude lower than estimates from Sachs and Repeta (1999), who calculated, using a two end member source model that 46–70 % of the nitrate pool in the eastern Mediterranean Sea originates from N<sub>2</sub> fixation. As pointed out by Krom et al. (2010), the main argument used in N stable isotopic studies conducted in the Mediterranean Sea (Pantoja et al., 2002; Sachs and Repeta, 1999) is based on the depleted  $\delta^{15}\text{N}$  of deep-water nitrate, PON and chlorophyll, but these mass balance calculations did not take into account the atmospheric deposition of NO<sub>3</sub><sup>−</sup>. These latter N inputs are however among the highest over the global ocean ( $75 \text{ mmol m}^{-2} \text{ yr}^{-1}$ , Krom et

**Table 3.** Vertical eddy diffusivity coefficient ( $K_z$ , in  $\text{m}^{-2} \text{d}^{-1}$ ) measured by Cuypers et al. (2011) at every LD station using a SCAMP microprofiler ( $K_z$  values given for stations 15, 19 and 24 are estimated as the average  $K_z$  of all stations),  $\text{NO}_3^-$  gradient at the nitracline ( $\mu\text{mol N m}^{-4}$ ) calculated by Moutin et al. (2011), and resulting vertical  $\text{NO}_3^-$  flux ( $\mu\text{mol N m}^{-2} \text{d}^{-1}$ ). Those fluxes have been calculated at the base of the euphotic zone (see Moutin et al., 2011 for euphotic layer depths).

Station	$K_z$ min ( $\text{m}^{-2} \text{d}^{-1}$ )	$K_z$ max ( $\text{m}^{-2} \text{d}^{-1}$ )	$\text{NO}_3^-$ gradient ( $\mu\text{mol m}^{-4}$ )	N flux min ( $\mu\text{mol N m}^{-2} \text{d}^{-1}$ )	N flux max ( $\mu\text{mol N m}^{-2} \text{d}^{-1}$ )
C	0.37	0.54	25	9	13
B	0.34	0.86	16	5	13
A	0.16	0.52	12	2	6
15	0.38	0.56	41	16	23
19	0.38	0.56	106	40	59
24	0.38	0.56	80	30	45

**Table 4.** Calculated external N inputs to the eastern and western Mediterranean basins without taking into account the exchanges at the straits and winter convection. All values are given in  $10^9$  moles  $\text{year}^{-1}$ .

Source (Eastern Med.)	N input ( $\times 10^9$ moles $\text{year}^{-1}$ )	Source
Atmospheric inputs (2002–2005)	107	Mihalopoulos, Unpub. Data
Riverine inputs	45	Ludwig et al. (2009)
Black Sea	8	Krom et al. (2004)
$\text{N}_2$ fixation	0.1–0.7	this study
Total inputs to the basin	160	
Source (Western Med.)	N input ( $\times 10^9$ moles $\text{year}^{-1}$ )	Source
Atmospheric inputs (2002–2005)	41	Markaki et al. (2009)
Riverine inputs	26	Ludwig et al. (2009)
$\text{N}_2$ fixation	2–18	this study
Total inputs to the basin	69–85	

al., 2004) and exhibit highly depleted  $\delta^{15}\text{N}-\text{NO}_3^-$  (−3.1 ‰, Mara et al., 2009). By including atmospheric deposition in the same isotopic mass balance calculation performed by Sachs and Repeta (1999) and Pantoja et al. (2002), Mara et al. (2009) could explain the unusually low  $\delta^{15}\text{N}$  without the need of  $\text{N}_2$  fixation. Recent studies conducted in the tropical North Atlantic confirmed that inputs of low  $\delta^{15}\text{N}-\text{NO}_3^-$  from the atmosphere have to be taken into account when investigating the present-day N-cycle in oceanic environments subjected to high atmospheric inputs (Baker et al., 2007; Knapp et al., 2005).

#### 4.2.2 Western Mediterranean Sea (Station B to A to Rhone river mouth)

$\text{N}_2$  fixation rates in the western basin were 10 to  $76 \mu\text{mol N m}^{-2} \text{d}^{-1}$ . They are in the same orders of magnitude than those commonly measured in the tropical and subtropical Atlantic and Pacific oceans (Table 2). The contribution of  $\text{N}_2$  fixation to new primary production also in-

creased to reach around 9 % at stations A and B and up to 17–35 % at stations 19 and 24 located further North-East (Table 1). Those numbers are in accordance with data obtained by Garcia et al. (2006) in the western basin (DYFAMED time series station), who showed that  $\text{N}_2$  fixation represents 27 % of new primary production during the stratification period, and can reach up to 55 % by the end of the summer. These numbers are also comparable to those obtained at station ALOHA (Karl et al., 1997) or in the equatorial Pacific Ocean (Bonnet et al., 2009), in which diazotrophs develop extensively, sustaining up to 50 % of new primary production. At station A and B, the vertical diffusive  $\text{NO}_3^-$  fluxes to the euphotic zone were 9–13 and 5–13  $\mu\text{mol m}^{-2} \text{d}^{-1}$ , respectively, and atmospheric deposition of nitrogen 16–34 and 10–27  $\mu\text{mol m}^{-2} \text{d}^{-1}$ , respectively (Table 1). Those numbers indicate that all nitrogen sources ( $\text{N}_2$  fixation,  $\text{NO}_3^-$  vertical fluxes and atmospheric deposition) contribute almost equally to sustain new primary production at those stations. Further west (stations 15, 19, 24), all N sources increase and  $\text{N}_2$  fixation seems to be the major process feeding new primary



production at station 24. It has to be noted that at some stations, the sum of all estimated sources of “new” N are not sufficient to sustain 100 % of new primary production. This may come from the possible underestimation of  $N_2$  fixation by the  $^{15}N_2$ -tracer addition technique (Montoya et al., 1996). Mohr et al. (2010) recently demonstrated that the  $^{15}N_2$  bubble injected in seawater does not attain equilibrium with surrounding water, leading to a  $^{15}N_2$  concentration lower than assumed in the  $^{15}N_2$ -fixation calculations. Moreover, estimated new primary production numbers (Table 1) are possibly overestimated; direct measurements are needed in the future to refine those calculations.

At the scale of the Western Mediterranean Sea, if we consider  $N_2$  fixation occurring six months per year at mean rates of  $10\text{--}76\ \mu\text{mol m}^{-2}\text{ d}^{-1}$  (this study), the net input of N through  $N_2$  fixation represents  $2\text{--}18 \times 10^9\text{ mol N}$  (Table 4), which is up to 20–45 times higher than the annual N supply by  $N_2$  fixation for the eastern basin. Moreover, if we consider that the N supply by both atmospheric deposition (Markaki et al., 2010) and rivers (Ludwig et al., 2009) are greatly reduced compared to the eastern basin (Table 4), the contribution of  $N_2$  fixation to total N supply is reinforced, reaching up to 25 % of external N inputs in the western basin (excluding exchanges at the straits and winter convection) compared to 0.4 % maximum in the eastern basin.

The decrease of  $N_2$  fixation from West to East is probably due to decreasing phosphate availability towards the eastern Mediterranean Sea. Phosphate turn over time is the first indicator of phosphate availability in oligotrophic marine areas where phosphate concentrations are close to the detection limit of chemical measurements (Moutin et al., 2008). From experimental work carried out in the South West Pacific ocean, a critical phosphate turn over times  $> 50\text{ h}$  was determined for *Trichodesmium* spp. to grow (Moutin et al., 2005). Uniform phosphate turn over times  $< 10\text{ h}$  were measured in surface waters during the BOUM cruise (Mauriac et al., 2011), which may prevent *Trichodesmium* spp. and maybe other  $N_2$ -fixing organisms with high energetic request, to develop extensively. These latter authors showed a clear deepening of the phosphate turn over time isoline of  $10\text{ h}$  (they are  $< 10\text{ h}$  above this isoline) from 50–60 m-depth in the western basin, to 100–120 m in the eastern basin, indicating much lower phosphate availability in the eastern basin. Ridame et al. (2011) confirmed that  $N_2$  fixation was clearly limited by phosphate availability at the time of the cruise (Ridame et al., 2011). Dissolved iron availability did not seem to play a key role in controlling  $N_2$  fixation in the Mediterranean Sea at the time of the cruise (Ridame et al., 2011), as dissolved iron concentrations are relatively high both in the eastern (Statham and Hart, 2005) and the western (Bonnet and Guieu, 2006) basins, and have been seen to be controlled by atmospheric deposition during the stratification period (Bonnet and Guieu, 2006).

Interestingly, the highest  $N_2$  fixation rates across the BOUM transect were not measured in nitrate-depleted wa-

ters, but rather in water masses exhibiting the highest nitrate concentrations ( $0.15\text{--}2.00\ \mu\text{M}$ ), close to the Rhone river plume. Because diazotrophic cyanobacteria have the ability to fix  $N_2$ ,  $NO_3^-$ -rich waters have traditionally been considered prohibitive for  $N_2$  fixation (Carpenter, 1983). In particular, since breaking the triple bond  $N_2$  molecule is energetically expensive, it has been assumed that if N in the form of  $NO_3^-$  and/or  $NH_4^+$  is present, the assimilation of these forms of N will be used before  $N_2$  fixation occurs (Falkowski, 1983; Karl et al., 2002). However, our understanding of marine nitrogen fixation is constantly evolving and increasing numbers of field studies report the presence of nitrogen fixation and/or active diazotrophs in a wide range of N-rich ecosystems, including relatively high latitudes (Needoba et al., 2007), nutrient enriched estuarine and coastal waters (Short and Zehr, 2007; Rees et al., 2009; Grosse et al., 2010; Bombar et al., 2011), upwelling area (Moutin et al., 2008), eddies (Church et al., 2009) and even High Nutrient, Low Chlorophyll waters (Bonnet et al., 2009). In particular,  $N_2$  fixation seems to be particularly favoured as soon as N:P ratios drop below 16:1, even if nitrate concentrations are high, like above large OMZs” (e.g. Capone and Knapp, 2007) (e.g. Capone and Knapp, 2007).  $N_2$  fixation in *Trichodesmium erythraeum* and *Crocospaera watsonii* cultures appears to be sensitive to N:P ratios (A. Knapp, personal communication). Other recent culture data confirmed that micromolar levels ( $10\ \mu\text{M}$ ) of nitrate do not inhibit  $N_2$  fixation of the unicellular diazotrophic cyanobacterium *Crocospaera* (Dekaezemacker and Bonnet, In revision) and inhibits only partly  $N_2$  fixation for *Trichodesmium erythraeum* (Holl and Montoya, 2005). Interestingly, the highest  $N_2$  fixation rates to date reported in the Mediterranean Sea were measured at the time series station DYFAMED in March, at the end of the spring bloom ( $400\ \mu\text{mol m}^{-2}\text{ d}^{-1}$ , Garcia et al., 2006) and in October ( $1700\ \mu\text{mol m}^{-2}\text{ d}^{-1}$ , D. G. Capone, personal communication, 2006), when the stratification breaks down and surface waters start to be enriched in nutrients. In both cases, nitrate were not depleted (around  $0.5\text{--}1\ \mu\text{mol l}^{-1}$ ), but N:P ratios were way below 16:1. Those  $N_2$  fixation rates are greatly higher than those reported in this study for the stratification period, and indicate that budgets calculated in Table 4 (based only on planktonic fluxes measured during the stratification period) could potentially be revised upward. Moreover, a recent methodological study indicates that the  $^{15}N_2$  method used in most of studies dedicated to  $N_2$  fixation do underestimate  $N_2$  fixation (Mohr et al., 2010). Finally,  $N_2$  fixation associated to seagrasses needs to be better estimated, as well as  $N_2$  fixation performed by heterotrophic bacteria in the aphotic zone. These latter data are contrary to our previous thinking that  $N_2$  fixation is maximum during the stratification period characterized by nitrate-depleted surface waters. These results potentially have important biogeochemical repercussions as  $N_2$  fixation occurring in environments or seasons characterized by high nitrate concentrations are poorly taken into account in biogeochemical N budgets.

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