Planktonic dinitrogen fixation in the Mediterranean Sea: a major biogeochemical process during the stratified period?

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Abstract

This study provides extensive data on planktonic N\textsubscript{2} fixation fluxes across the whole Mediterranean Sea, representing a variety of trophic conditions. They show that N\textsubscript{2} fixation occurs in Mediterranean waters during the stratification period, with a clear decreasing trend from the western basin (10–76 µmol m\textsuperscript{-2} d\textsuperscript{-1}) to the eastern basin (0–0.4 µmol m\textsuperscript{-2} d\textsuperscript{-1}). Highest rates are measured in the less oligotrophic areas, between the surface and 75 m-depth, and 45 to 75% of N\textsubscript{2} fixation are performed within the picoplanktonic fraction (<3 µm). While the biogeochemical impact of N\textsubscript{2} fixation in the eastern basin seems negligible, N\textsubscript{2} fixation is able to sustain up to 35% of new primary production during the stratified period and accounts for up to 25% of the external “new” N supply to the western basin during that period. These data disagree with indirect estimates of N\textsubscript{2} fixation based on geochemical tracers and nutrient budgets, who suspected N\textsubscript{2} fixation to increase with increasing N/P ratios and decreasing stable N isotopic signature of particulate organic nitrogen and NO\textsubscript{3}\textsuperscript{-} from west to east. These results finally point out the need to assess N\textsubscript{2} fixation at other seasons characterized by less oligotrophic 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 NO\textsubscript{3}\textsuperscript{-} and PO\textsubscript{4}\textsuperscript{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, which have been very useful for geochemical studies to trace some biological processes occurring in the ocean, and their evolution over large time and space scales (e.g. Gruber and Sarmiento, 1997).
The Mediterranean Sea is a stimulating case study 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 (McGill, 1965, 1969; Coste and Minas, 1967; Bethoux et al., 1998; Krom et al., 1991; Moutin and Raimbault, 2002; Pujo-Pay et al., 2010). 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 over 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 is a semi enclosed basin receiving among the highest rates of aeolian material deposition in the global ocean (Guerzoni et al., 1999). In addition to these pulses of mineral dust, it continuously receives anthropogenic aerosols from industrial and domestic activities from populated areas around the basin and other parts of Europe that form a background over the Mediterranean Sea (Chester et al., 1996; Guieu et al., 1997). These atmospheric inputs provide dissolved nitrogen and phosphorus to the surface Mediterranean waters 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 provides nutrients in a ratio greatly in excess to 16:1 (Krom et al., 2004).

The second hypothesis generally preferred in the literature argue that high N/P ratios are due to intense dinitrogen (N$_2$) fixation, the prokaryotic-mediated conversion of atmospheric dinitrogen into the bioavailable form of ammonia. This process provides a source of N, non 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\textsubscript{2} fixation in the present and recent geological times. Pan-
toja et al. (2002) revealed an eastward decrease in surface δ\textsuperscript{15}N (‰) suspended particulate organic nitrogen (PON) (2.7 ± 1.2‰ to −0.2 ± 0.7‰), δ\textsuperscript{15}N, chlorophyll a (2.6 ± 2.3‰ to −7.1 ± 1.3‰) and deep-water nitrate (3.4 ± 0.5‰ to 2.5 ± 0.1‰), im-
plying an eastward increase in the contribution of N\textsubscript{2} fixation to the water column N budget. They estimated that N\textsubscript{2} fixation accounted for 20 to 90% of the N supply to the western and eastern Mediterranean, respectively, exceeding previous estimates (7–40% according to the hypothesis considered) 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\textsubscript{2}-fixing organisms, some free trichomes of \textit{Trichodesmium} spp. have been detected in the 60’s (Margalef, 1969), but large blooms of \textit{Trichodesmium} spp. have never been observed in the Mediterranean Sea, probably because phosphate availability never reach 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 affiliated to Archeae, Proteobacteria and/or Cyanobacteria (Man-Aharonovich et al., 2007; Le Moal and Biegala, 2009), and to the filamentous \textit{Richelia intracellularis} (Bar Zeev et al., 2008) in low abundances. The potential of these organisms to bloom and fix dini-
trogen 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 and controversal (Krom et al., 2010) N\textsubscript{2} fixation fluxes at one isolated station in the Levan-
tine basin (Rees et al., 2006), or extremely low values at six stations distributed over the basin (Ibello et al., 2010), leading 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\textsubscript{2} uptake measurements over a large number of stations (17) along a 2000 km transect in the Mediterranean Sea (Fig. 1), exhibiting strong trophic gradients. The objectives of
this study were (i) to quantify N$_2$ fixation rates along the transect, (ii) to determine the relative importance of the large (> 3 µm) and the small (< 3 µm) 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.

2 Material and methods

This research was carried out in the Mediterranean Sea onboard the R/V Atalante in the framework of the BOUM project. The transect started in La Seyne sur Mer, France (43°07′ N, 05°52′ E) on 16 June 2008, stretched to the eastern basin (42°50′ N, 38°50′ E) and ended in La Seyne sur Mer (43°07′ N, 05°52′ E) on 20 July (Fig. 1).

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 CTDO 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). These latest 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) at day 1 and day 3. For each SD station, they were performed only at two depths (50% and 3% surface light levels, corresponding to the subsurface and the upper 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% HCl) 4.5 L polycarbonate bottles equipped with septum caps to which additions of 4 mL of $^{15}$N$_2$ gas (99%, EURISOTOP) were made using a gas-tight syringe. Just before dawn and immediately after tracer addition, incubations were carried out for 24 h. They were performed in situ at LD stations on a drifting mooring line situated at the same depth from which the samples were collected, and in on-deck incubators equipped with circulating seawater at the specified irradiances using blue screening at SD stations. Incubations were terminated by gently filtering samples; at SD stations, for each depth, samples were filtered under low vacuum pressure ($< 100 \text{ mm Hg}$) onto pre-combusted (4 h at 450°C) GF/F filters (25 mm diameter, 0.7 µm pore size) for determination of the “bulk” nitrogen fixation. At LD stations, 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 µm polycarbonate filters (washed onto a GF/F pre-combusted (4 h at 450°C) filters) for fraction $> 3 \mu m$, while the filtrate was collected onto a pre-combusted GF/F filter for the $< 3 \mu m$ fraction (0.7 µm pore size). Sample filters were stored in 2 ml glass tubes and dried at 60°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 $^{15}$N atom% as a function of increasing particulate nitrogen mass on both natural and $^{15}$N enriched material, and the constant response of $^{15}$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. $^{15}$N atom% was linear (Fisher test, $p < 0.01$) between 0.20
and 39 µmol N, which is within the range of particulate nitrogen measured in all our samples (0.45 to 3.65 µmoles 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 µmoles, and quantification limits ranged 0.10 to 0.19 µmoles N. The $^{15}$N isotope enrichment of a sample was calculated using the $^{15}$N atom% excess over time, over the $^{15}$N atom% in samples taken from the same station at time zero (background $\delta^{15}$N). The value of time zero enrichment was determined on bottles filtered immediately after adding $^{15}$N$_2$. We considered the results to be significant when $^{15}$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%).

### 3 Results

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

The global range of N$_2$ fixation rates measured over the entire Mediterranean transect was 0.10–1.80 nmol l$^{-1}$ d$^{-1}$. They exhibited strong meridional gradients (Fig. 2), the lowest rates being measured in the eastern basin, averaging 0.43 ± 0.33 nmol l$^{-1}$ d$^{-1}$. Fluxes increased toward the western basin (average: 0.63 ± 0.45 nmol l$^{-1}$ d$^{-1}$) to reach maximum values in the Rhone river plume, with rates reaching 1.80 ± 0.19 nmol l$^{-1}$ 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 (0.10–0.33 nmol l$^{-1}$ d$^{-1}$) and A (0.19–0.43 nmol l$^{-1}$ d$^{-1}$) located in the center and the western Mediterranean Sea.

Data indicate that rates were globally 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, at least at the surface and above the DCM+, where data were available for SD stations.

Data from the three vertical profiles obtained at LD stations A, B and C (Fig. 3) indicate that rates were not uniform over the vertical. 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 fluxes 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 fluxes at this intermediate depth (around 50 m-depth) over the euphotic zone.

Depth integrated rates were calculated at stations # A, B, C, 15, 19, 24, Table 1; data confirmed that integrated rates globally decreased from the western basin (10.2 ± 2.7 to 76.2 ± 7.7 μmol l$^{-1}$ d$^{-1}$ at station B and 27, respectively, Table 1) to the eastern basin (0.4 ± 0.1 μmol l$^{-1}$ d$^{-1}$ at station C). Size fractionation experiments performed at LD stations (Fig. 3) indicate that a significant part of the nitrogen fixation fluxes were associated with the picoplanktonic size fraction (fraction < 3 μm), 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 trend over the vertical at station B, where the maximum fluxes were located between the surface and 50 m-depth. At station A, maximum fluxes associated to 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 dataset 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 basin during the
Planktonic dinitrogen fixation in the Mediterranean Sea

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Introduction

Data report that N\textsubscript{2} fixation is an active process at that period along the transect, with rates ranging from 0.10–1.80 nmol l\textsuperscript{-1} d\textsuperscript{-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. (2010), who used whole-cell hybridization of specific Nitro821 oligonucleotide probe to detect and quantify unicellular diazotrophic cyanobacteria (UCYN\textsubscript{2}-Fix) in three size fractions over the same transect. They report that UCYN\textsubscript{2}-Fix were recovered at all stations across the entire Mediterranean 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\textsubscript{2} fixation rates were recovered in the picoplanktonic size fraction (<3 µm), which is in accordance with data from Le Moal et al. (2010), showing that the diazotrophic community was dominated at 99.9% by picoplanktonic diazotrophic cyanobacteria. Further \textit{nif}H analysis performed on the picoplanktonic fraction revealed however that clones libraries were dominated at 90% by sequences belonging to diazotrophic proteobacteria, while the remaining 10% clustered with the UCYN-A, indicating that these two groups of organisms must be responsible for the fluxes recovered in <3 µm fraction.

N\textsubscript{2} fixation rates measured in the size fraction >3 µm could also be due to picoplanktonic UCYN\textsubscript{2}-Fix, as Le Moal et al. (2010) have shown that 25% of this community was recovered within the 3–10 µm and >10 µm 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 UCYN\textsubscript{2}-Fix were recovered in large size fractions, indicating that these unicellular forms may be responsible for some rates formally attributed to organisms >3 µm such as \textit{Trichodesmium} spp. or \textit{Richelia} spp. Nonetheless, phytoplankton net hauls revealed the presence of the filamentous cyanobacterium \textit{Richelia intracellulraris} at all stations sampled across the entire basin during the BOUM cruise (Crombet et al., 2010). They were in association with the centric diatoms...
Hemiaulus hauckii and Rhizosolenia styliformis and must contribute to the $N_2$ fixation fluxes measured in the size fraction $>3\mu m$ in this study. Crombet et al. (2010) 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, and could also be located above. Richelia in association with Hemiaulus is thus suspected here (along with possible picoplanktonic UCYN$_2$-Fix associated to nano- and micro-particles) to be responsible for the $N_2$ fixation fluxes measured at depth (50–60 m), especially at stations #17 to 19 around 80 m-depth, where they reached a maximum of 250 cells l$^{-1}$ (Gomez et al., personal communication, 2010), corresponding to $N_2$ fixation fluxes of 0.51 to 0.72 nmol l$^{-1}$ d$^{-1}$, and at stations located in the Rhone river plume where they reach up to 150 cells l$^{-1}$, corresponding to $N_2$ fixation fluxes of 1.06 to 1.80 nmol l$^{-1}$ d$^{-1}$. This type of association between Richelia and centric diatoms has already been observed in eastern Mediterranean waters during a two-years time series survey performed off the coast of Israel (Bar Zeev et al., 2008); the authors report the presence of Richelia throughout the year, with small peaks in autumn ($\sim 50$ heterocysts l$^{-1}$), coinciding with the deepening of the mixed layer depth, but never observed large blooms ($>1000$ heterocysts l$^{-1}$), like the ones observed in the tropical Atlantic ocean (e.g. Villareal, 1994; Carpenter et al., 1999), possibly due to the lack of essential nutrients such as phosphate (Moutin et al., 2008). Finally, we cannot exclude the contribution of Trichodesmium spp. to the $N_2$ fixation fluxes measured in the $>3\mu m$ 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., 2010).

4.2 Biogeochemical significance of $N_2$ fixation in the Mediterranean Sea

4.2.1 Eastern Mediterranean Sea (Stations B to C)

This study indicates that $N_2$ fixation fluxes decreased when going eastward, indicating potential contrasted behaviours between the western and the eastern basins. In the eastern basin, $N_2$ fixation rates were extremely low, especially at station C where
they reached 0.4 ± 0.1 µmol m⁻² d⁻¹, which is in agreement with recent data obtained by Ibello et al. (2010) who also reported extremely low fluxes (0.5 to 2 µmol m⁻² d⁻¹) at three stations in the Levantine basin in May–June 2007. These low rates 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 µmol m⁻² d⁻¹, Yogev et al., 2011), as well as by the annual N₂ fixation cycle performed by Bar Zeev et al. (2008). All these concordant low fluxes in the eastern Mediterranean Sea are extremely low compared to measurements performed in the tropical and subtropical Atlantic and Pacific Oceans (Table 2), indicating a potential nutrient and/or temperature limitation for the extensive development of N₂-fixing organisms. These low N₂ fixation rates in the eastern basin relative to the western basin disagree with previous isotopic data (Pantoja et al., 2002) reporting an eastward increase in the contribution of N₂ 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 N₂ fixation. On the basis of a C/N = 6.6, the contribution of N₂ fixation to the nitrogen demand of “new” primary production (considered as 10% of primary production measured in situ using ¹⁴C following 24-h incubation duration (Christaki et al., 2010) at station C is negligible (0–0.3%, Table 1), indicating on the opposite a minor contribution of N₂ fixation at the studied period.

As a first approximation, anticyclonic eddies may be considered as a closed systems. Then, in addition to N₂ fixation, the only sources of “new” nitrogen to the euphotic zone during intense summer stratification are vertical diffusive fluxes from below and atmospheric deposition. The vertical NO₃⁻ flux in the euphotic layer was calculated from the product of the vertical eddy diffusivity coefficient (Kz, in m² s⁻¹) measured at every LD station using a SCAMP microprofiler (Cuypers et al., 2010), and the NO₃⁻ gradient at the nitracline following calculations detailed in Moutin et al. (2010) (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., 2010), to which we applied a mean
deposition velocity of particles of 0.5 to 1 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 µmol m\(^{-2}\) d\(^{-1}\), and total atmospheric dissolved nitrogen deposition of 10–27 µmol m\(^{-2}\) 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\(_2\) 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\(_3^-\) 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 (Kz) during the cruise, while Moutin and Raimbault (2002) estimated the Kz 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 Kz may be refined in the future using a larger number of SCAMP profiles to catch the variability associated to 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\(_2\) fixation occurs 6 months per year with mean areal rates of 0.4–3.0 µmol N m\(^{-2}\) d\(^{-1}\) (This study, Ibello et al., 2010; Yogev et al., 2011), the total N supply provided by N\(_2\) fixation to the eastern Mediterranean Sea is 0.1–0.7 \times 10^9 mol N year\(^{-1}\) (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 mol N year\(^{-1}\)), 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 N₂ 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 δ¹⁵N of deep-water nitrate, PON and chlorophyll, but these mass balance calculations did not take into account the atmospheric deposition of NO₃⁻. These latest N inputs are however among the highest over the global ocean (75 mmol m⁻² y⁻¹, Krom et al., 2004) and exhibit highly depleted δ¹⁵N(NO₃⁻)(−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 δ¹⁵N without the need of N₂ fixation. Recent studies conducted in the tropical North Atlantic confirmed since ever that inputs of low δ¹⁵N(NO₃⁻) from the atmosphere have to be taken into account when investigating the present-day N-cycle in oceanic environments submitted 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)

N₂ fixation rates clearly increased when going to the western basin to reach areal rates of 10 to 76 µmol N m⁻² d⁻¹. Those fluxes 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 N₂ fixation to new primary production also increased 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 N₂ 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 NO$_3^-$ fluxes to the euphotic zone were 9–13 and 5–13 µmol m$^{-2}$ d$^{-1}$, respectively, and atmospheric deposition of nitrogen 16–34 and 10–27 µmol m$^{-2}$ d$^{-1}$, respectively (Table 1). Those numbers indicate that all nitrogen sources (N$_2$ fixation, 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 N$_2$ fixation seems to be the major process feeding new primary production at station 24. However, primary production and $K_z$ values at those three western stations were estimated (Tables 1 and 3) and not measured, so the NO$_3^-$ fluxes and new primary production numbers would need to be refined using direct measurements.

At the scale of the Western Mediterranean Sea, if we consider N$_2$ fixation occurring six months per year at mean rates of 10–76 µmol m$^{-2}$ d$^{-1}$ (this study), the net input of N through N$_2$ fixation represents 2–18 x 10$^9$ 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.

4.3 Longitudinal variability of N$_2$ fixation and potential controlling factors

N$_2$ fixation decreased from West to East, probably due to lower phosphate availability when going eastward, as shown by decreasing phosphate turn over times (i.e. the ratio between natural phosphate concentration and uptake) in the upper waters (Moutin et al., 2002; Mauriac et al., 2010). 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 h was determined for *Trichodesmium* spp. to grow (Moutin et al., 2005). Phosphate turn over times < 10 h were measured in surface waters during the BOUM cruise (Mauriac et al., 2010), which may prevent *Trichodesmium* spp. and maybe other N₂-fixing organisms with high energetic request, to develop extensively.

Ridame et al. (2010) confirmed that N₂ fixation was clearly limited by phosphate availability at the time of the cruise, despite stimulation was higher with Saharan dust (Ridame et al., 2010). Dissolved iron availability did not seem to play a key role in controlling N₂ fixation in the Mediterranean Sea at the time of the cruise (Ridame et al., 2010), 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 N₂ fixation rates across the BOUM transect were not measured in nitrate-depleted waters, but rather in water masses exhibiting the highest nitrate concentrations (0.15–2.00 µM), close to the Rhone river plume. Because diazotrophic cyanobacteria have the ability to fix N₂, NO₃⁻-rich waters have traditionally been considered prohibitive for N₂ fixation (Carpenter, 1983). In particular, since breaking the triple bond N₂ molecule is energetically expensive, it has been assumed that if N in the form of NO₃⁻ and/or NH₄⁺ is present, the assimilation of these forms of N will be used before N₂ 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), upwelling area (Moutin et al., 2008), eddies (Church et al., 2009) and even High Nutrient, Low Chlorophyll waters (Bonnet et al., 2009). In particular, N₂ fixation seems to be particularly favoured as soon as N/P ratios drop below 16:1, even if nitrate concentrations are high, as observed above large OMZs (e.g. Capone and Knapp, 2007). This sensitivity of N₂ fixation to N/P ratios has recently been confirmed
in cultures of *Trichodesmium erythraeum* and *Crocosphaera watsonii* (Knapp et al., personal communication, 2010). Other recent culture data recently confirmed that micromolar levels (10 µM) of nitrate do not inhibit N₂ fixation of the unicellular diazotrophic cyanobacterium *Crocosphaera* (Dekaezemacker and Bonnet, 2011) and inhibits only partly N₂ fixation for *Trichodesmium erythraeum* (Holl and Montoya, 2005). Interestingly, the highest N₂ 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 µmol m⁻² d⁻¹, Garcia et al., 2006) and in October (1700 µmol m⁻² d⁻¹, Capone et al., personal communication, 2007), when the stratification breaks down and surface waters start to be enriched in nutrients. In both cases, nitrate were not depleted (around 0.5–1 µmol l⁻¹), but N/P ratios were way below 16:1. Those N₂ 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, N₂ fixation associated to seagrasses needs to be better estimated, as well as N₂ fixation performed by heterotrophic bacteria in the aphotic zone. These latest data are contrary to our previous thinking that N₂ fixation is maximum during the stratification period characterized by nitrate-depleted surface waters. These results potentially have important biogeochemical repercussions as N₂ fixation occurring in environments or seasons characterized by high nitrate concentrations are poorly taken into account into biogeochemical N budgets.

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References


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Dekaezemacker, J. and Bonnet, S.: Sensitivity analysis of nitrogen fixation to combined nitrogen forms (NO₃⁻ and NH₄⁺) in two strains of the marine diazotroph Crocosphaera watsonii (Cyanobacteria), Mar. Ecol. Prog. Ser., under review, 2011.


Le Moal, M. and Biegala, I.: Diazotrophic unicellular cyanobacteria in the northwestern Medi-
Planktonic dinitrogen fixation in the Mediterranean Sea

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Table 1. Integrated primary production, N₂ 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 µmol N m⁻² d⁻¹) has been considered as 10% of total PP (Moutin and Raimbault, 2002).

<table>
<thead>
<tr>
<th>Location</th>
<th>Primary production*</th>
<th>N₂ fixation</th>
<th>% New PP</th>
<th>Vertical nitrate diffusion</th>
<th>% New PP</th>
<th>N atmospheric deposition</th>
<th>% New PP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg C m⁻² d⁻¹</td>
<td>µmol N m⁻² d⁻¹</td>
<td></td>
<td>µmol N m⁻² d⁻¹</td>
<td></td>
<td>µmol N m⁻² d⁻¹</td>
<td></td>
</tr>
<tr>
<td>St A</td>
<td>156–164</td>
<td>10.2 ± 2.7/14.8 ± 1.5</td>
<td>6–9</td>
<td>9–13</td>
<td>5–8</td>
<td>16–34</td>
<td>9–20</td>
</tr>
<tr>
<td>St B</td>
<td>187–195</td>
<td>11.8 ± 5.6/18.6 ± 4.6</td>
<td>7–9</td>
<td>5–13</td>
<td>7–25</td>
<td>10–27</td>
<td>5–13</td>
</tr>
<tr>
<td>St C</td>
<td>137–240</td>
<td>0.4 ± 0.1/0 ± 0</td>
<td>0–0.3</td>
<td>2–6</td>
<td>0.1–4</td>
<td>10–27</td>
<td>4–18</td>
</tr>
<tr>
<td>St 15</td>
<td>200–400</td>
<td>24.6 ± 3.8</td>
<td>6–11</td>
<td>16–23</td>
<td>4–11</td>
<td>60**</td>
<td>14–28</td>
</tr>
<tr>
<td>St 19</td>
<td>200–400</td>
<td>35.9 ± 4</td>
<td>8–17</td>
<td>40–59</td>
<td>14–18</td>
<td>60**</td>
<td>15–28</td>
</tr>
<tr>
<td>St 24</td>
<td>200–400</td>
<td>76.2 ± 7.8</td>
<td>17–35</td>
<td>30–45</td>
<td>7–21</td>
<td>60**</td>
<td>16–28</td>
</tr>
</tbody>
</table>

* Data from Christaki et al. (2010) 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.
Table 2. Range of oceanic \( N_2 \) fixation areal rates measured in some contrasted oceanic environments.

<table>
<thead>
<tr>
<th>Location</th>
<th>Areal rates ( \mu \text{mol m}^{-2} \text{d}^{-1} )</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tropical North Pacific (ALOHA)</td>
<td>69</td>
<td>Dore et al. (2002)</td>
</tr>
<tr>
<td>Tropical Atlantic</td>
<td>86</td>
<td>Goering et al. (1966)</td>
</tr>
<tr>
<td>Tropical Atlantic</td>
<td>24</td>
<td>Voss et al. (2004)</td>
</tr>
<tr>
<td>Tropical Atlantic</td>
<td>140</td>
<td>Voss et al. (2004)</td>
</tr>
<tr>
<td>Eastern Tropical North Pacific</td>
<td>520</td>
<td>Montoya et al. (2004)</td>
</tr>
<tr>
<td>Equatorial Pacific</td>
<td>18–358</td>
<td>Bonnet et al. (2009)</td>
</tr>
<tr>
<td>Bermuda</td>
<td>41–93</td>
<td>Orcutt et al. (2001)</td>
</tr>
<tr>
<td>Arabian Sea</td>
<td>35–99</td>
<td>Capone et al. (1998)</td>
</tr>
<tr>
<td>North East Pacific (California)</td>
<td>0–15</td>
<td>Needoba et al. (2007)</td>
</tr>
<tr>
<td>Eastern Mediterranean Sea</td>
<td>0–0.4</td>
<td>This study</td>
</tr>
<tr>
<td>Western Mediterranean Sea</td>
<td>10–76</td>
<td>This study</td>
</tr>
</tbody>
</table>
Table 3. Vertical eddy diffusivity coefficient ($K_z$, in m$^2$ d$^{-1}$) measured by Cuypers et al. (2010) 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), NO$_3^-$ gradient at the nitracline (µmol N m$^{-4}$) calculated by Moutin et al. (2010), and resulting vertical NO$_3^-$ flux (µmol N m$^{-2}$ d$^{-1}$). Those fluxes have been calculated at the base of the euphotic zone (see Moutin et al., 2010 for euphotic layer depths). $K_z$ values given at stations 15, 19 and 24 estimated as the mean of all $K_z$ values measured over the cruise.

<table>
<thead>
<tr>
<th>Station</th>
<th>$K_z$ min (m$^2$ d$^{-1}$)</th>
<th>$K_z$ max (m$^2$ d$^{-1}$)</th>
<th>NO$_3^-$ gradient (µmol m$^{-4}$)</th>
<th>N flux min (µmol N m$^{-2}$ d$^{-1}$)</th>
<th>N flux max (µmol N m$^{-2}$ d$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>0.37</td>
<td>0.54</td>
<td>25</td>
<td>9</td>
<td>13</td>
</tr>
<tr>
<td>B</td>
<td>0.34</td>
<td>0.86</td>
<td>16</td>
<td>5</td>
<td>13</td>
</tr>
<tr>
<td>A</td>
<td>0.16</td>
<td>0.52</td>
<td>12</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>15</td>
<td>0.38</td>
<td>0.56</td>
<td>41</td>
<td>16</td>
<td>23</td>
</tr>
<tr>
<td>19</td>
<td>0.38</td>
<td>0.56</td>
<td>106</td>
<td>40</td>
<td>59</td>
</tr>
<tr>
<td>24</td>
<td>0.38</td>
<td>0.56</td>
<td>80</td>
<td>30</td>
<td>45</td>
</tr>
</tbody>
</table>
**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 year$^{-1}$.

<table>
<thead>
<tr>
<th>Source</th>
<th>N input (x $10^9$ moles year$^{-1}$)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Eastern Med.)</td>
<td></td>
<td>(Western Med.)</td>
</tr>
<tr>
<td>Riverine inputs</td>
<td>45</td>
<td>Ludwig et al. (2009)</td>
</tr>
<tr>
<td>Black Sea</td>
<td>8</td>
<td>Krom et al. (2004)</td>
</tr>
<tr>
<td>$N_2$ fixation</td>
<td>0.1–0.7</td>
<td>This study</td>
</tr>
<tr>
<td>Total inputs to the basin</td>
<td>160</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Source</th>
<th>N input (x $10^9$ moles year$^{-1}$)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Western Med.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Riverine inputs</td>
<td>26</td>
<td>Ludwig et al. (2009)</td>
</tr>
<tr>
<td>$N_2$ fixation</td>
<td>2–18</td>
<td>This study</td>
</tr>
</tbody>
</table>
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.
Fig. 2. Longitudinal cross section of N$_2$ fixation rates (nmol l$^{-1}$ d$^{-1}$) along the BOUM 10 transect (0–160 m) including fourteen SD stations and the three LD stations. x-axis: distance 11 from the station 27.
Fig. 3. Vertical profiles of $N_2$ fixation fluxes (nmol l$^{-1}$ d$^{-1}$) measured at LD stations A (a), B (b) and C (c). Dark blue 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 ($<3$ µm) fraction. Error bars represent uncertainties on the measurement. Green circle: Chl a concentration ($\mu$g l$^{-1}$).