Biogeosciences Discuss., 8, 1197–1225, 2011
www.biogeosciences-discuss.net/8/1197/2011/
doi:10.5194/bgd-8-1197-2011

Biogeosciences Discussions

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

S. Bonnet, O. Grosso, and T. Moutin

INSU-CNRS, Laboratoire d'Océanographie Physique et Biogéochimique (LOPB), UMR6535, CNRS-IRD-Université de la Méditerranée, Centre d'Océanologie de Marseille, Faculté des Sciences de Luminy, Case 901, 13288 Cedex 09 Marseille, France

Received: 3 February 2011 – Accepted: 4 February 2011 – Published: 10 February 2011

Correspondence to: S. Bonnet (sophie.bonnet@univmed.fr)

Published by Copernicus Publications on behalf of the European Geosciences Union.

Abstract

This study provides extensive data on planktonic N_2 fixation fluxes across the whole Mediterranean Sea, representing a variety of trophic conditions. They show that N_2 fixation occurs in Mediterranean waters during the stratification period, with a clear decreasing trend from the western basin (10–76 µmol m^{-2} d^{-1}) to the eastern basin (0–0.4 µmol m^{-2} d^{-1}). Highest rates are measured in the less oligotrophic areas, between the surface and 75 m-depth, and 45 to 75% of N_2 fixation are performed within the picoplanktonic fraction (< 3 µm). 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 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_2 fixation based on geochemical tracers and nutrient budgets, who suspected N_2 fixation to increase with increasing N/P ratios and decreasing stable N isotopic signature of particulate organic nitrogen and NO_3^- from west to east. These results finally point out the need to assess N_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_3^- and 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, 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).

BGD
8, 1197–1225, 2011

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 (Mc Gill, 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-meditated 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

BGD
8, 1197–1225, 2011

evidence for significant N_2 fixation in the present and recent geological times. Pantoja et al. (2002) revealed an eastward decrease in surface $\delta^{15}N$ (‰) suspended particulate organic nitrogen (PON) (2.7 ± 1.2‰ to -0.2 ± 0.7‰), $\delta^{15}N$, chlorophyll *a* (2.6 ± 2.3‰ to -7.1 ± 1.3‰) and deep-water nitrate (3.4 ± 0.5‰ to 2.5 ± 0.1‰), 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% 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_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 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 *Richelia intracellularis* (Bar Zeev et al., 2008) in low abundances. 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 and controversial (Krom et al., 2010) N_2 fixation fluxes at one isolated station in the Levantine 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 Ultraoligotrophic Mediterranean, June–July 2008), we had the opportunity to perform N_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

BGD
8, 1197–1225, 2011

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

BGD
8, 1197–1225, 2011

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 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 µm, while the filtrate was collected onto a pre-combusted GF/F filter for the < 3 µm 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

BGD
8, 1197–1225, 2011

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

BGD
8, 1197–1225, 2011

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

BGD
8, 1197–1225, 2011

stratification period. Data report that N_2 fixation is an active process at that period along the transect, with rates ranging from 0.10–1.80 nmol l^{-1} 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. (2010), who used whole-cell hybridization of specific Nitro821 oligonucleotide probe to detect and quantify unicellular diazotrophic cyanobacteria (UCYN$_2$-Fix) in three size fractions over the same transect. They report that UCYN$_2$-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_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 *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_2 fixation rates measured in the size fraction > 3 µm could also be due to picoplanktonic UCYN$_2$-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$_2$-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 *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., 2010). They were in association with the centric diatoms

BGD
8, 1197–1225, 2011

Hemiaulus hauckii and *Rhizosolenia styliformis* and must contribute to the N_2 fixation fluxes measured in the size fraction > 3 µm 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 (~ 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 µm 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

BGD
8, 1197–1225, 2011

they reached $0.4 \pm 0.1\,\mu mol\,m^{-2}\,d^{-1}$, which is in agreement with recent data obtained by Ibello et al. (2010) who also reported extremely low fluxes (0.5 to $2\,\mu mol\,m^{-2}\,d^{-1}$) 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 $3\,\mu mol\,m^{-2}\,d^{-1}$, Yogev et al., 2011), as well as by the annual N_2 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 N_2-fixing organisms. These low N_2 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 N_2 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_2 fixation. On the basis of a C/N = 6.6, the contribution of N_2 fixation to the nitrogen demand of "new" primary production (considered as 10% of primary production measured in situ using ^{14}C 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 N_2 fixation at the studied period.

As a first approximation, anticyclonic eddies may be considered as a closed systems. Then, in addition to N_2 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 NO_3^- flux in the euphotic layer was calculated from the product of the vertical eddy diffusivity coefficient (*Kz*, in $m^2\,s^{-1}$) measured at every LD station using a SCAMP microprofiler (Cuypers et al., 2010), and the NO_3^- 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

BGD
8, 1197–1225, 2011

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 (ε) and the buoyancy frequency *N(z)* according to Osborn (1980). However, those direct measurements of ε 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 × 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 × 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

BGD
8, 1197–1225, 2011

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_2 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 $\delta^{15}N$ of deep-water nitrate, PON and chlorophyll, but these mass balance calculations did not take into account the atmospheric deposition of NO_3^-. 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 $\delta^{15}N\text{-}NO_3^-$ (−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 $\delta^{15}N$ without the need of N_2 fixation. Recent studies conducted in the tropical North Atlantic confirmed since ever that inputs of low $\delta^{15}N\text{-}NO_3^-$ from the atmosphe 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)

N_2 fixation rates clearly increased when going to the western basin to reach areal rates of 10 to 76 $\mu mol\,N\,m^{-2}\,d^{-1}$. 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 N_2 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 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,

BGD
8, 1197–1225, 2011

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 *Kz* 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 × 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

BGD
8, 1197–1225, 2011

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 N_2-fixing organisms with high energetic request, to develop extensively.

Ridame et al. (2010) confirmed that N_2 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 N_2 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 N_2 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 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), 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, as observed above large OMZs (e.g. Capone and Knapp, 2007). This sensitivity of N_2 fixation to N/P ratios has recently been confirmed

BGD
8, 1197–1225, 2011

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 N_2 fixation of the unicellular diazotrophic cyanobacterium *Crocosphaera* (Dekaezemacker and Bonnet, 2011) 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 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 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, 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 latest 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 into biogeochemical N budgets.

Acknowledgements. This is a contribution of the BOUM (Biogeochemistry from the Oligotrophic to the Ultraoligotrophic Mediterranean) experiment (http://www.com.univ-mrs.fr/BOUM) of the french national LEFE-CYBER program, the European IP SESAME and the international IMBER project. The BOUM experiment was coordinated by the Institut des Sciences de l'Univers (INSU) and managed by the Centre National de la Recherche Scientifique (CNRS). The authors thank the crew of the R/V *l'Atalante* for outstanding shipboard operations and P. Raimbault for his help on the cruise preparation and constructive exchanges on isotopic analyses. C. Marec and L. Prieur are warmly thanked for their efficient help in CTD rosette

BGD
8, 1197–1225, 2011

management and data processing as well as Marie Paule Torre for the LEFE CYBER database management. L. Prieur as also thanked for his careful advices on the manuscript.

The publication of this article is financed by CNRS-INSU.

References

Baker, A. R., Weston, K., Kelly, S. D., Voss, M., Streu, P., and Cape, J. N.: Dry and wet deposition of nutrients from the tropical Atlantic atmosphere: links to primary productivity and nitrogen fixation, Deep-Sea Res. Part I, 54, 1704–1720, 2007.

Bar Zeev, E., Yogev, T., Man-Aharonovich, D., Kress, N., Herut, B., Béjà O., and Berman-Frank, I.: Seasonal Dynamics of the Endosymbiotic, Nitrogen-fixing Cyanobacterium *Richelia intracellularis* in the Eastern Mediterranean Sea, The ISME Journal, 2, 911–923, 2008.

Béthoux, J. P. and Copin-Montégut, G.: Biological fixation of atmospheric nitrogen in the Mediterranean Sea, Limnol. Oceanogr., 31, 1353–1358, 1986.

Béthoux, J. P., Morin, P., Chaumery, C., Connan, O., and Ruiz-Pino, D.: Nutrient in the Mediterranean Sea, mass balance and statistical analysis of concentrations with respect to environmental change, Marine Chemistry, 63, 155–169, 1998.

Bonnet, S. and Guieu, C.: Atmospheric Forcing on the Annual Iron Cycle in the Mediterranean Sea. A one-year Survey, J. Geophys. Res., 111, C09010, doi:10.1029/2005JC003213, 2006.

Bonnet, S., Biegala, I., Dutrieux, P., Slemons, L. O., and Capone, D. G.: Nitrogen fixation in the western equatorial Pacific, Global Biogeochem. Cyc., 23, GB3012, doi:10.1029/2008GB003439, 2009.

Capone, D. G. and Knapp, A.: A marine nitrogen cycle fix?, Nature, 445, 159–160, 2007.

Capone, D. G., Subramaniam, A., Montoya, J. P, Voss, M., Humborg, C., Johansen, A., Siefert, R., and Carpenter, E. J.: An extensive bloom of the N2-fixing cyanobacterium, *Tri-*

BGD
8, 1197–1225, 2011

chodesmium erythraeum, in the central Arabian Sea, Mar. Ecol. Prog. Ser., 172, 281–292, 1998.
Carpenter, E. J.: Physiology and ecology of marine planktonic *Trichodesmium*, Mar. Biol. Lett., 4, 69–16, 1983.
Carpenter, E. J., Montoya, J. P., Burns, J., Mulholland, M. R., Subramaniam, A., and Caone, D. G.: Extensive bloom of a N_2-fixing diatom/cyanobacterial association in the tropical Atlantic Ocean, Mar. Ecol. Prog. Ser., 185, 273-283, 1999.
Chester, R., Nimmo, M., and Keyse, S.: The influence of Saharan and Middle Eastern desert-derived dust on the trace metal composition of Mediterranean aerosols and rainwater: An overview, in: The Impact of Desert Dust Across the Mediterranean, edited by: Guerzoni, S. and Chester, R., 253– 273, Springer, New York, 1996.
Church, M. J., Mahaffey, C., Letelier, R. M., Lukas, R., Zehr, J. P., and Karl, D. M.: Physical forcing of nitrogen fixation and diazotroph community structure in the North Pacific subtropical gyre, Global Biogeochem. Cy., 23, GB2020, doi:10.1029/2008GB003418, 2009.
Coste, B. and Minas, H.: Premières observations sur la distribution des taux de productivité et des concentrations en sels nutritifs des eaux de surface du golfe du Lion, Cahiers océanogr. CD.E.C., 19(5), 417–429, 1967.
Crombet, Y., Leblanc, K., Quéguiner, B., Moutin, T., Rimmelin, P., Ras, J., Claustre, H., Leblond, N., Oriol, L., and Pujo-Pay, M.: Deep silicon maxima in the stratified oligotrophic Mediterranean Sea, Biogeosciences Discuss., 7, 6789–6846, doi:10.5194/bgd-7-6789-2010, 2010.
Cuypers, Y., Bouruet-Aubertot, P., Fuda, J. L., Marec, C.: Turbulence in the upper ocean during BOUM experiment: direct measurements and finescale parameterization, Biogeosciences Discuss., in preparation, 2011.
Dekaezemacker, J. and Bonnet, S.: Sensitivity analysis of nitrogen fixation to combined nitrogen forms (NO_3^- and NH_4^+) in two strains of the marine diazotroph *Crocosphaera* watsonii (Cyanobacteria), Mar. Ecol. Prog. Ser., under review, 2011.
Dore, J. E., Brum, J. R., Tupas, L. M., and Karl, D. M.: Seasonal and interannual variability in sources of nitrogen supporting export in the oligotrophic subtropical North Pacific Ocean, Limnol. Oceanogr., 47, 1595–1607, 2002.
Falkowski, P.: Light-shade adaptation and vertical mixing of marine phytoplankton: A comparative field study, J. Mar. Res., 41, 215–237, 1983.
Garcia, N., Raimbault, P., Gouze, E., and Sandroni, V.: Fixation de diazote et production primaire en Mediterranee occidentale, C.R. Biologies, 329, 742-750, 2006.

BGD
8, 1197–1225, 2011

Gruber, N. and Sarmiento, J.: Global patterns of marine nitrogen fixation and denitrification, Glob. Biogeochem. Cycles., 11, 243–23, 1997.
Guerzoni, S., Chester, R., Dulac, F., Herut, B., Loye-Pilot, M. D., Measures, C., Migon, C., Molinaroli, E., Moulin, C., Rossini, P., Saydam, C., Soudine, A., and Ziveri, P.: The role of atmospheric deposition in the 5 biogeochemistry of the Mediterranean Sea, Prog. Oceanogr., 44, 147-190, 1999.
Guieu, C., Chester, R., Nimmo, M., Martin, J. M., Guerzoni, S., Nicolas, E., Mateu, J., and Keyse, S.: Atmospheric input of dissolved and particulate metals to the northwestern Mediterranean, Deep Sea Res.-Pt. II, 44, 655–674, 1997.
Holl, C. M. and Montoya, J. P.: Interactions between nitrate uptake and N_2 fixation in *Trichodesmium*, J. Phycol., 41, 1178–1183, 2008.
Ibello, V., Cantoni, C., Cozzi, S., and Civitarese, G.: First basin-wide experimental results on N_2 fixation in the open Mediterranean Sea, Geophys. Res. Lett., 37, L03608, doi:10.1029/2009GL041635, 2010.
Karl, D., Letelier, R., Tupas, L., Dore, J., Christian, I., and Hebel, D.: The role of nitrogen fixation 15 in biogeochemical cycling in the subtropical North Pacific Ocean, Nature, 388, 6642, 533-538, 1997.
Karl, D., Michaels, A., Bergman, B., Capone, D. G., Carpenter, R. C., Letelier, R., Lipschultz, F., Paerl, H. W., Sigman D. M., and Stal, L.: Dinitrogen fixation in the world's oceans, Biochemistry, 57–58, 47–98, 2002.
Knapp, A. N., Sigman, D. M., and Lipschultz, F.: N isotopic composition of dissolved organic nitrogen and nitrate at the Bermuda Atlantic Time-series Study site, Global Biogeochem. Cy., 19, GB1018, doi:10.1029/2004GB002320, 2005.
Krom, M. D., Kress, N., and Brenner, S.: Phosphorus limitation of primary productivity in the eastern Mediterranean Sea, Limnol. Oceanogr., 36, 424–432, 1991.
Krom, M. D., Herut, B., and Mantoura, R. F. C.: Nutrient budget for the Eastern Mediterranean: Implications for phosphorus limitation, Limnol. Oceanogr., 49(5), 1582–1592, 2004.
Krom, M. D., Emeis, K. C., and Van Cappellen, P.: Why is the Eastern Mediterranean phosphorus limited?, Prog. Oceanogr., 85(3–4), 236–244, 2010.
Langlois, R. J., Hümmer, D., and LaRoche, J.: Abundances and Distributions of the Dominant nifH 4 Phylotypes in the Northern Atlantic Ocean. Appl. Environ. Microbiol., 74(6), 1922–9, doi:10.1128/AEM.01720-07, 2008.
Le Moal, M. and Biegala, I.: Diazotrophic unicellular cyanobacteria in the northwestern Mediter-

BGD
8, 1197–1225, 2011

ranean Sea: a seasonal cycle, Limnol. Oceanogr., 2009, 54(3), 845–855, 2009.
Le Moal, M., Collin, H., and Biegala, I. C.: Intriguing diversity among diazotrophic picoplankton along a Mediterranean transect: from the origin of plastids to the dominance of rhizobia, Biogeosciences Discuss., 7, 8779–8816, doi:10.5194/bgd-7-8779-2010, 2010.
Ludwig, W., Dumont, E. Meybeck, M., and Heussner, S.: River discharges of water and nutrients to the Mediterranean and Black Sea: Major drivers for ecosystem changes during past and future decades?, Prog. Oceanogr., 80, 199–217, 2009.
Man-Aharonovich, D., Kress, N., Bar Zeev, E., Berman-Frank, I., and Béjà, O.: Molecular ecology of nifH genes and transcripts in Eastern Mediterranean Sea, Environ. Microbiol., 9, 2354–2363, 2007.
Mara, P., Mihalopoulos, N., Gogou, A., Daehnke, K., Schlarbaum, T., Emeis, K. C., and Krom, M.: Isotopic composition of nitrate in wet and dry atmospheric deposition on Crete in the eastern Mediterranean Sea, Global Biogeochem. Cy., 23, GB4002, doi:10.1029/2008GB003395, 2009.
Margalef, R.: Composición específica del fitoplancton de la costa catalano-levantina (Mediterráneo occidental) en 1962–1967, Inv. Pesq., 33, 345–380, 1969.
Markaki, Z., Oikonomou, K., Kocak, M., Kouvarakis, G., Chaniotaki, A., Kubilay, N., and Mihalopoulos, N.: Atmospheric deposition of inorganic phosphorus in the Levantine Basin, Eastern Mediterranean: spatial and temporal variability and its role in seawater productivity, Limnol. Oceanogr., 48(4), 1557–1568, 2003.
Markaki, Z., Loÿe- Pilot, M. D., Violaki, K., and Mihalopoulos, N.: Variability of atmospheric deposition of dissolved nitrogen and phosphorus in the Mediterranean and possible link to the anomalous seawater N/P ratio, Marine Chemistry, 120, 187–194, 2010.
Mauriac, R., Moutin, T., and Baklouti, M.: Accumulation of DOC in Low Phosphate Low Chlorophyll (LPLC) area: is it related to higher production under high N:P ratio?, Biogeosciences Discuss., 7, 7091–7130, doi:10.5194/bgd-7-7091-2010, 2010.
McGill, D. A.: A preliminary study of the oxygen and phosphate distribution in the Mediterranean Sea, Deep-Sea Res., 8, 259–275, 1961.
Montoya, J. P., Holl, C., Zehr, J. P., Hansen, A., Villareal, T., and Capone D. G.: High rates of N_2 fixation by unicellular diazotrophs in the oligotrophic Pacific Ocean, Nature, 430, 1027–1032, 2004.
Montoya, J. P., Voss, M., Kahler P., and Capone, D. G.: A simple, high-precision, high-sensitivity tracer assay for N_2 fixation, Appl. Environ. Microbiol., 62, 986–993, 1996.

BGD
8, 1197–1225, 2011

Moutin, T. and Raimbault, P.: Primary production, carbon export and nutrients availability in western and eastern Mediterranean Sea in early summer 1996, MATER Special Issue, J. Mar. Syst., 33–34, 273–288, 2002.

Moutin, T., Thingstad, T. F., Van Wambeke, F., Marie, D., Slawyk, G., Raimbault, P., and Claustre, H.: Does competition for nano-molar phosphate supply explain the predominance of the cyanobacterium Synechococcus?, Limnol. Oceanogr., 47, 1562–1567, 2002.

Moutin, T., Van Den Broeck, N., Beker, B., Dupouy, C. Rimmelin, P. and Le Bouteiller, A.: Phosphate availability controls *Trichodesmium* spp. biomass in the SW Pacific ocean, Mar. Ecol. Progress.-Ser., 297, 15–21, 2005.

Moutin, T., Karl, D. M., Duhamel, S., Rimmelin, P., Raimbault, P., Van Mooy, B. A. S., and Claustre, H.: Phosphate availability and the ultimate control of new nitrogen input by nitrogen fixation in the tropical Pacific Ocean, Biogeosciences, 5, 95–109, doi:10.5194/bg-5-95-2008, 2008.

Moutin, T., Van Wambeke, F., and Prieur, L.: Introduction to the Biogeochemistry from the Oligotrophic to the Ultraoligotrophic Mediterranean (BOUM) experiment, Biogeosciences Discuss., in preparation, 2011.

Needoba, J. A., Foster, R. A., Sakamoto, C., Zehr, J. P., and Johnson, K. S.: Nitrogen fixation by unicellular diazotrophic cyanobacteria in the temperate oligotrophic North Pacific Ocean, Limnol. Oceanogr., 52, 1317–1327, 2007.

Orcutt, K. M., Lipschultz, F., Gundersen, K., Arimoto, R., Michaels, A. F., Knap, A. H., Gallon, J. R.: A seasonal study of the significance of N_2 fixation by *Trichodesmium* spp. at the Bermuda Atlantic Time-series Study (BATS) site, Deep-Sea Res. Part II: Topical Studies in Oceanography, 48(8–9), 1583–1608, 2001.

Pantoja, S., Repeta, D. J., Sachs, J. P., Sigman, D. M.: Stable isotope constraints on the nitrogen cycle of the Mediterranean Sea water column, Deep Sea Res.-Pt. I, 49(9), 1609–1621, 2002.

Pujo-Pay, M., Conan, P., Oriol, L., Cornet-Barthaux, V., Falco, C., Ghiglione, J.-F., Goyet, C., Moutin, T., and Prieur, L.: Integrated survey of elemental stoichiometry (C, N, P) from the Western to Eastern Mediterranean Sea, Biogeosciences Discuss., 7, 7315–7358, doi:10.5194/bgd-7-7315-2010, 2010.

Redfield, A. C.: On the proportions of organic derivations in sea water and their relation to the composition of plankton, in: James Johnstone Memorial Volume, edited by: Daniel, R. J., University Press of Liverpool, 177–192, 1934.

BGD

8, 1197–1225, 2011

Rees, A. P., Law, C. S., and Woodward, E. M. S.: High rates of nitrogen fixation during an in-situ phosphate release experiment in the Eastern Mediterranean Sea, Geophys. Res. Lett., 33, L10607, doi:10.1029/2006GL025791, 2006.

Rees, A. P., Gilbert, J. A., and Kelly-Gerreyn, B. A.: Nitrogen fixation in the western English Channel (NE Atlantic ocean), Mar. Ecol-Progr. Ser., 374, 7-12, doi:10.3354/meps07771, 2009.

Ridame, C., Le Moal, M., Guieu, C., Ternon, E., Biegala, I., L'Helguen, S., and Pujo-Pay, M.: Nutrient control of N2 fixation and growth of unicellular diazotrophs in the oligotrophic Mediterranean Sea and potential impact from a Saharan dust event, Biogeosciences Discuss., in preparation, 2011.

Short, S. and Zehr, J. P.: Nitrogenase gene expression in the Chesapeake Bay Estuary, Environ Microbiol., 9, 1591–1596, 2007.

Statham, P. J. and Hart, V.: Dissolved iron in the Cretan Sea (eastern Mediterranean), Limnol. Oceanogr., 50(4), 1142–1148, 2005.

Sacchs, J. P. and Repeta, D.: Oligotrophy and nitrogen fixation during eastern Mediterranean sapropel events, Science, 286, 2485–2488, 1999.

Ternon, E., Guieu, C., Ridame, C., L'Helguen, S., and Catala, P.: Longitudinal variability of the biogeochemical role of Mediterranean aerosols in the Mediterranean Sea, Biogeosciences Discuss., 7, 8087–8121, doi:10.5194/bgd-7-8087-2010, 2010.

Villareal, T. A.: Widespread occurrence of the Hemiaulus-cyanobacterial symbiosis in the southwest North Atlantic Ocean, Bull. Mar. Sci., 54(1), 1–7, 1994.

Voss, M., Croot, P., Lochte, K., Mills, M., and Peeken, I.: Patterns of nitrogen fixation along 10° N in the tropical Atlantic, Geophys. Res. Lett., 31, L23S09, doi:10.1029/2004GL020127, 2004.

Yogev, T., Rahav, E., Bar Zeev, E., Man-Aharonovich, D., Kress, N., Béjà, O., Mulholland, M., Herut, B., Berman-Frank, I.: Is dinitrogen fixation significant in the Levantine Basin, Eastern Mediterranean Sea?, Environ. Microbiol., 1–19, doi:10.1111/j.1462-2920.2010.02402.x, 2011.

BGD
8, 1197–1225, 2011

Table 1. Integrated primary production, N_2 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 $\mu mol\,N\,m^{-2}\,d^{-1}$) has been considered as 10% of total PP (Moutin and Raimbault, 2002).

Location	Primary production* $mg\,C\,m^{-2}\,d^{-1}$	N_2 Fixation $\mu mol\,N\,m^{-2}\,d^{-1}$	% New PP	Vertical nitrate diffusion $\mu mol\,N\,m^{-2}\,d^{-1}$	% New PP	N atmospheric deposition $\mu mol\,N\,m^{-2}\,d^{-1}$	% New PP
St A	156–164	10.2 ± 2.7/14.8 ± 1.5	6–9	9–13	5–8	16–34	9–20
St B	187–195	11.8 ± 5.6/18.6 ± 4.6	7–9	5–13	7–25	10–27	5–13
St C	137–240	0.4 ± 0.1/0 ± 0	0–0.3	2–6	0.1–4	10–27	4–18
St 15	200–400	24.6 ± 3.8	6–11	16–23	4–11	60**	14–28
St 19	200–400	35.9 ± 4	8–17	40–59	14–18	60**	15–28
St 24	200–400	76.2 ± 7.8	17–35	30–45	7–21	60**	16–28

* 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.

BGD
8, 1197–1225, 2011

Table 2. Range of oceanic N_2 fixation areal rates measured in some contrasted oceanic environments.

Location	Areal rates $\mu mol\, m^{-2}\, d^{-1}$	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	140	Voss et al. (2004)
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	35–99	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

BGD
8, 1197–1225, 2011

Table 3. Vertical eddy diffusivity coefficient (*Kz*, in $m^2\,d^{-1}$) measured by Cuypers et al. (2010) at every LD station using a SCAMP microprofiler (*Kz* values given for stations 15, 19 and 24 are estimated as the average *Kz* of all stations), NO_3^- gradient at the nitracline ($\mu mol\,N\,m^{-4}$) calculated by Moutin et al. (2010), and resulting vertical NO_3^- flux ($\mu mol\,N\,m^{-2}\,d^{-1}$). Those fluxes have been calculated at the base of the euphotic zone (see Moutin et al., 2010 for euphotic layer depths). *Kz* values given at stations 15, 19 and 24 estimated as the mean of all *Kz* values measured over the cruise.

Station	*Kz* min ($m^2\,d^{-1}$)	*Kz* max ($m^2\,d^{-1}$)	NO_3^- gradient ($\mu mol\,m^{-4}$)	N flux min ($\mu mol\,N\,m^2\,d^{-1}$)	N flux max ($\mu mol\,N\,m^2\,d^{-1}$)
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

BGD
8, 1197–1225, 2011

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 109 moles year^{-1}.

Source (Eastern Med.)	N input (x 10^9 moles year^{-1})	Source
Atmopsheric inputs (2002–2005)	107	Mihalopoulos, Unpub. Data
Riverine inputs	45	Ludwig et al. (2009)
Black Sea	8	Krom et al. (2004)
N_2 fixation	0.1–0.7	This study
Total inputs to the basin	160	

Source (Western Med.)	N input (x 10^9 moles year^{-1})	Source
Atmopsheric inputs (2002–2005)	41	Markaki et al. (2009)
Riverine inputs	26	Ludwig et al. (2009)
N_2 fixation	2–18	This study

BGD
8, 1197–1225, 2011

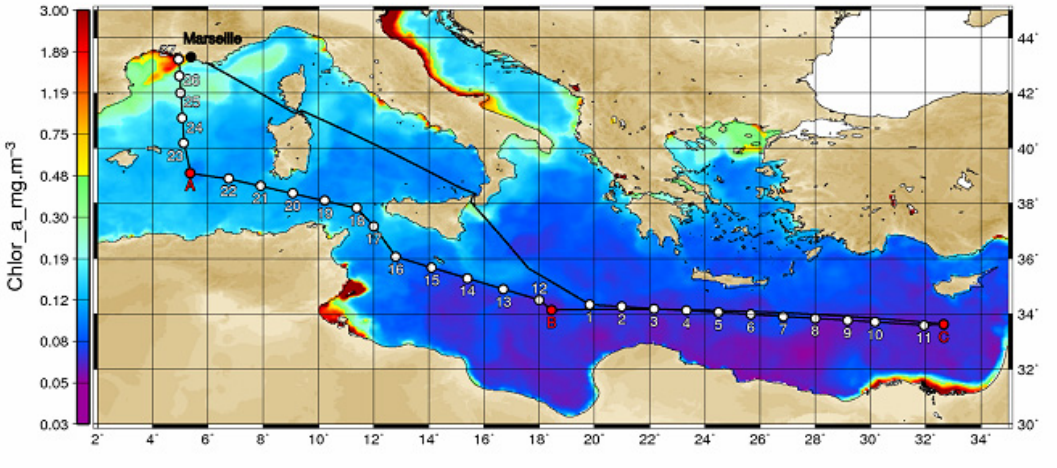

Fig. 1. Transect of the BOUM cruise superimposed on a SeaWiFS surface Chl-a composite image (June 2008), and location of the fourteen short (numbers) and three LD stations (letters) of the cruise.

BGD
8, 1197–1225, 2011

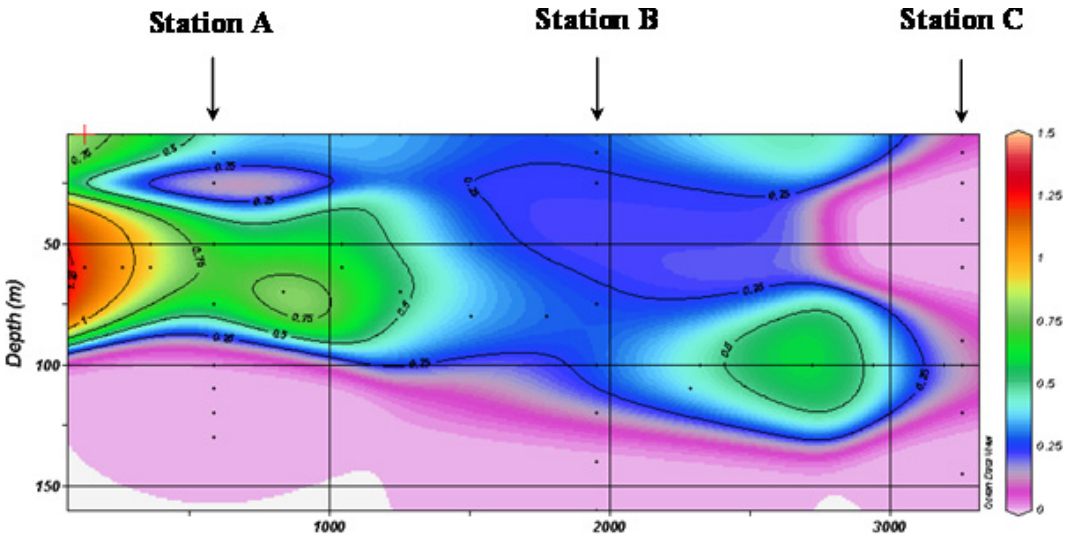

Fig. 2. Longitudinal cross section of N_2 fixation rates (nmol l^{-1},d^{-1}) along the BOUM 10 transect (0–160 m) including fourteen SD stations and the three LD stations. x-axis: distance 11 from the station 27.

BGD
8, 1197–1225, 2011

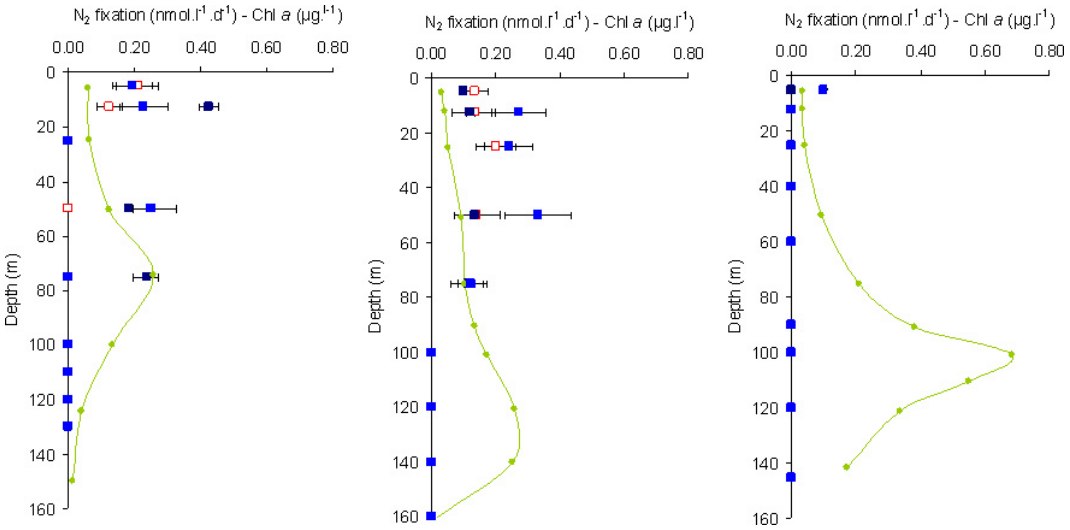

Fig. 3. Vertical profiles of N_2 fixation fluxes (nmol l^{-1} d^{-1}) measured at LD stations A **(a)**, B **(b)** and C **(c)**. Dark blue squares: bulk N_2 fixation measured at day 1. Light blue squares: bulk N_2 fixation measured at day 3 at the same station. Red squares: N_2 fixation in the picoplanktonic (< 3 µm) fraction. Error bars represent uncertainties on the measurement. Green circle: Chl *a* concentration (µg l^{-1}).

BGD
8, 1197–1225, 2011