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Eyal Rahav

Barak Herut

Noga Stambler

Edo Bar-Zeev

Margaret R. Mulholland
Old Dominion University, mmulholl@odu.edu

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Uncoupling between dinitrogen fixation and primary productivity in the eastern Mediterranean Sea

Eyal Rahav,[1] Barak Herut,[2] Noga Stambler,[1] Edo Bar-Zeev,[1] Margaret R. Mulholland,[3] and Ilana Berman-Frank[1]

[1]   In the nitrogen (N)-impoverished photic zones of many oceanic regions, prokaryotic organisms fixing atmospheric dinitrogen (N_2; diazotrophs) supply an essential source of new nitrogen and fuel primary production. We measured dinitrogen fixation and primary productivity (PP) during the thermally stratified summer period in different water regimes of the oligotrophic eastern Mediterranean Sea, including the Cyprus Eddy and the Rhodes Gyre. Low N_2 fixation rates were measured (0.8–3.2 μmol N m^{-2} d^{-1}) excluding 10-fold higher rates in the Rhodes Gyre and Cyprus Eddy (~20 μmol N m^{-2} d^{-1}). The corresponding PP increased from east to west (200–2500 μmol C m^{-2} d^{-1}), with relatively higher productivity recorded in the Rhodes Gyre and Cyprus Eddy (2150 and 2300 μmol C m^{-2} d^{-1}, respectively). These measurements demonstrate that N_2 fixation in the photic zone of the eastern Mediterranean Sea contributes only negligibly by direct inputs to PP (i.e., cyanobacterial diazotrophs) and is in fact uncoupled from PP. By contrast, N_2 fixation is significantly coupled to bacterial productivity and to net heterotrophic areas, suggesting that heterotrophic N_2 fixation may in fact be significant in this ultraoligotrophic system. This is further substantiated by the high N_2 fixation rates we measured from aphotic depths and by the results of phylogenetic analysis in other studies showing an abundance of heterotrophic diazotrophs.

1.   Introduction

[2]   The Mediterranean Sea is the largest semienclosed marine basin in the world, and it is characterized by low dissolved inorganic concentrations of nitrogen (N) and phosphorus (P) [*Krom et al.*, 1991; *Bethoux et al.*, 1992; reviewed in *Krom et al.*, 2010] that are rapidly taken up and recycled by phytoplankton and bacteria [*Psarra et al.*, 2005; *Thingstad et al.*, 2005; *Tanaka et al.*, 2007, 2011]. The increasing gradient of subsurface inorganic N and P concentrations from east to west greatly influences the photosynthetic biomass [*Moutin and Raimbault*, 2002; *Krom*, 2004]. Chlorophyll concentrations and primary productivity (PP) reflect this geographical gradient and increase from east to west [*D'Ortenzio and Ribera D'Alcala*, 2009], with the lowest chlorophyll and primary production found in the Levantine basin of the eastern Mediterranean Sea especially during the thermally stratified summer period [*Yacobi et al.*, 1995; *Gotsis-Skretas et al.*, 1999; *Bosc et al.*, 2004; *Ignatiades et al.*, 2009; reviewed in *Siokou-Frangou et al.*, 2010, and *Berman-Frank and Rahav*, 2012].

[3]   Dissolved inorganic phosphorus has traditionally been considered the limiting nutrient for phytoplankton, especially in the eastern Mediterranean basin [*Krom et al.*, 1991; *Zohary and Robarts*, 1998; *Van Wambeke et al.*, 2002; *Tanaka et al.*, 2009]. However, limitation of primary and bacterial production by dissolved inorganic nitrogen availability as well as co-limitation of dissolved inorganic nitrogen and phosphorus also occur [*Thingstad et al.*, 2005; *Tanaka et al.*, 2011]. The limited availability of dissolved inorganic nitrogen sources in the upper photic zone and diazotrophic access to an almost infinite supply of atmospheric dinitrogen (N_2) could thus provide diazotrophs with a competitive edge in this environment.

[4]   Although not limited by nitrogen (N) as many marine autotrophs are, diazotrophs require dissolved inorganic phosphorus, dissolved Fe, and other micronutrients to supply their cellular demand for the metabolically expensive process of N_2 fixation [*Postgate*, 1979]. In many oceanic areas, diazotrophy is limited by dissolved inorganic phosphorus [*Sanudo-Wilhelmy et al.*, 2001; *Wu et al.*, 2003; *Moutin et al.*, 2008; *Moore et al.*, 2009], dissolved Fe [*Rueter*, 1988; *Kustka et al.*, 2003; *Capone et al.*, 1998], or both [*Mills et al.*, 2004; see review in *Sohm et al.*, 2011; *Zehr and Kudela*, 2011].

[1]Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat Gan, Israel.
[2]Israel Oceanographic and Limnological Research, National Institute of Oceanography, Haifa, Israel.
[3]Department of Ocean, Earth, and Atmospheric Sciences, Old Dominion University, Norfolk, Virginia, USA.

Corresponding author: I. Berman-Frank, Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat Gan 52900, Israel. (ilana.berman-frank@biu.ac.il)

[5] N$_2$ fixation further controls total primary production [*Falkowski*, 1997] as the supply of new nitrogen to the N-poor surface waters of the ocean by diazotrophs usually stimulates both autotrophic and heterotrophic communities of the planktonic and microbial food webs [*Carpenter and Capone*, 2008]. Thus, N$_2$ fixation should be positively correlated to primary production in two ways: (1) directly via photoautotrophic diazotrophs currently assumed to be the dominant component of the diazotrophic community in the photic zone, with sunlight providing the required energy for the energetically expensive process of N$_2$ fixation, and (2) indirectly via the contribution and recycling of the products of this "new N" (i.e., assimilated N, ammonia leakage, breakdown of diazotrophic blooms, etc.) stimulating primary production by non-diazotrophic photoautotrophs.

[6] In the ultraoligotrophic eastern Mediterranean Sea, very low rates of N$_2$ fixation have been measured in the photic layer over the past 6 years [*Ibello et al.*, 2010; *Bonnet et al.*, 2011; *Yogev et al.*, 2011]. The regulating factors leading to these low N$_2$ fixation rates have not yet been elucidated as dissolved Fe sources are assumed sufficient in the eastern Mediterranean Sea [*Statham and Hart*, 2005; *Theodosi et al.*, 2010], while nutrient enrichment studies including P, Fe, and Saharan dust yielded variable responses of N$_2$ fixation in different stations [*Ridame et al.*, 2011]. Diazotroph characterization via molecular phylogenetic identification indicates that many of the *nifH* sequences are homologous with heterotrophic bacterioplankton rather than with phototrophic cyanobacteria [*Man-Aharonovich et al.*, 2007; *Yogev et al.*, 2011].

[7] In this study, we evaluated the contribution and relationship of N$_2$ fixation from the photic layer to primary production in different water regimes of the eastern Mediterranean Sea. To this end, we measured in situ N$_2$ and carbon fixation from different depths within the photic zone and performed on-board microcosm experiments in 11 different locations throughout the Levantine basin over three consecutive years (2007–2009) during the stratified period.

2. Materials and Methods

2.1. Sampling Locations

[8] Water samples were collected on board the R/V *Mediterranean Explorer* for stations located within the Cyprus Eddy and Rhodes Gyre as well as pelagic stations uninfluenced by eddy circulation (Stations 2, 6, and 10) during the ISRLEV transect (July 2009) and on board R/V *Shikmona* for Stations H06, IL04, IL13, and IL16 during the SESAME Levantine basin transect (September 2008) [see *Yogev et al.*, 2011, for details]. Stations T1 and T2 off the Israeli coastline were also routinely sampled for 3 years [see *Yogev et al.*, 2011, for details]. Note that stations uninfluenced by the Cyprus Eddy and Rhodes Gyre are hereafter referred to as "background" stations. All sampling locations are illustrated in Figure 1, and the exact locations are specified in Table 1.

[9] Seawater was sampled using Niskin bottles (8 L) mounted on a rosette equipped with a CTD (Seabird 19 Plus), a fluorometer (Turner designs, Cyclops-7) for real-time chlorophyll *a* evaluation, and a PAR sensor (Seabird 19 Plus).

2.2. Inorganic Nutrients

[10] Duplicate water samples were collected in 15 mL acid-washed plastic scintillation vials. Nutrients were determined using a segmented flow Technicon AutoAnalyzer II system as described by *Krom et al.* [1991] and *Kress and Herut* [2001]. The precision values of nitrate + nitrite, phosphate, and silicic acid measurements were 0.02, 0.003, and 0.06 μM, respectively. The limit of detection (two times the standard deviation of the blank) was 0.075 μM for nitrate + nitrite, 0.008 μM for phosphate, and 0.03 μM for silicic acid.

[11] For phosphorus enrichment experiments, orthophosphate (PO_4^{-3}) solution (Sigma) was added into acid-clean 4.5 L polycarbonate Nalgene bottles, bringing the water to a final concentration of 100 nM P.
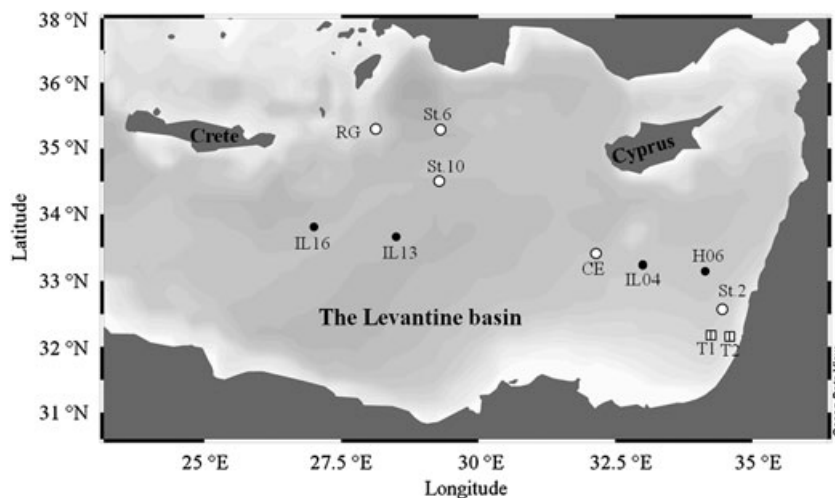

Figure 1. Map showing station locations of the ISRALEV cruise during July 2009 (white circles), stations sampled during the SESAME cruise (September 2008) (black circles), and stations sampled monthly off the Israeli coastal shelf (T1 and T2) during the stratified period (square symbols).

Table 1. Summary of the Sampling Locations and Dates at the Upper 150 m[a]

Station	Latitude (N)	Longitude (E)	Cruise	Sampling Date	Temperature (°C)	Salinity	NO$_2$ + NO$_3$ (mM m^{-2})	PO$_4$ (mM m^{-2})
2	32.5	34.4	ISRLEV	July 2009	17.2–28.3	39.3–39.5	28	2.9
Cyprus Eddy	33.4	32.1	ISRLEV	July 2009	17.2–26.7	39.3–39.4	26	2.8
6	35.3	29.3	ISRLEV	July 2009	15.7–25.7	39.1–39.5	33	2.3
10	34.5	29.3	ISRLEV	July 2009	15.5–26.3	39.1–39.5	53	2.6
Rhodes Gyre	35.3	28.1	ISRLEV	July 2009	14.9–23.6	39.0–39.4	136	2.8
H06	33.1	34.1	SESAME	September 2008	16.7–28.7	39.2–39.8	34	1.5
IL04	33.2	33.0	SESAME	September 2008	16.9–28.4	39.3–39.7	15	1.6
IL13	33.6	28.5	SESAME	September 2008	16.2–26.9	39.1–39.4	36	2.3
IL16	33.8	27.0	SESAME	September 2008	15.6–26.0	39.0–39.4	28	5.8
T1	32.2	34.5	Routine	Averaged summer	18.1–27.7	39.3–39.7	21	1.9
T2	32.2	34.2	Routine	Averaged summer	18.1–27.6	39.3–39.6	23	1.9

[a]Temperature and salinity are presented as ranges and inorganic nutrients (NO$_2$ + NO$_3$ and PO$_4$) are shown as integrated concentrations.

2.3. Dinitrogen Fixation

[12] Rates of N$_2$ fixation were measured on field samples using the ^{15}N$_2$ assimilation technique described by *Montoya et al.* [1996] and *Mulholland et al.* [2006]. Water was added into 4.5 L polycarbonate Nalgene bottles, sealed with septum tops, and spiked with 9 mL of ^{15}N$_2$ (99%). For each sampling depth, triplicate bottles were spiked with ^{15}N$_2$ and triplicate bottles were incubated with the natural abundance of N$_2$. All bottles were incubated on deck under ambient surface seawater temperatures and covered with neutral density screening adjusted to simulate light levels at the sampling depth. According to experience in the eastern Mediterranean Sea [*Bar-Zeev et al.*, 2008; *Yogev et al.*, 2011], incubations were 24–30 h in duration. Incubations were terminated by filtration onto precombusted 25 mm GF/F filters. Filters were dried overnight (~10 h) at 60°C and analyzed on a Europa 20/20 mass spectrometer equipped with an automated nitrogen and carbon analyzer preparation module. For isotope ratio mass spectrometry, standard curves to determine N and C mass were done with each sample run. Samples were run only when standard curves had R^2 values >0.99. At masses >4.7 μg N, the precision for the atom percent ^{15}N measurement was ±0.0001% based on daily calibrations made in association with sample runs and calibrations averaged over runs made over several years. For most of the results reported here, the masses were >4.7 μg N. However, samples with <4.7 μg N were only used if the precision was 0.0001% for that sample run. Standard masses ranged from 1.2 to 100 μg N and from 9.4 to 800 μg C. In addition to daily standard curves, reference standards and standards run as samples were run every six to eight samples. N$_2$ fixation rates were calculated using a mixing model [*Montoya et al.*, 1996] with the N solubility factors from *Weiss* [1970]. Based on natural abundance, N mass on the filters, incubation times, and precision of the mass spectrometer, our calculated detection limit for ^{15}N uptake was 0.02 nmol L^{-1} d^{-1} [*Yogev et al.*, 2011]. Integrated areal N$_2$ fixation rates were calculated from surface down to 150 m at all stations (4–6 m depths for each station in total).

2.4. Primary Productivity

[13] Photosynthetic carbon fixation rates were estimated using ^{13}C [*Mulholland and Bernhardt*, 2005]. Water samples were placed in triplicate clear 4.5 L polycarbonate Nalgene bottles, amended with (99%) NaH^{13}CO$_3$ (Sigma) to obtain 1% of the ambient dissolved inorganic carbon, and incubated under the same conditions and bottles as those applied in the ^{15}N incubations described above (n = 3 for

each depth). Triplicate parallel dark bottles were also incubated and subtracted from the light bottles to correct for dark carbon fixation. PP was integrated over 24 h. Incubations were terminated and analyzed as described above for the ^{15}N$_2$ uptake. The contribution of N$_2$ fixation to the N demand for PP was estimated using the measured particulate C/N ratio obtained for each sample.

2.5. Bacterial Productivity

[14] Bacterial productivity was estimated using the ^{3}H-leucine (Amersham; specific activity: 160 Ci mmoL^{-1}) incorporation method [*Simon et al.*, 1990; *Simon et al.*, 1992]. Triplicate (1.7 mL) samples were incubated with 200 nmol L^{-1} ^{3}H-leucine for 4–8 h at in situ temperatures in the dark. Preliminary experiments indicated that this was a saturating level of ^{3}H-leucine and that incorporation was linear during this time period (data not shown). Triplicate trichloroacetic acid (TCA)–killed samples served as controls. The incubations were terminated with the addition of 100 μL of cold TCA (100%) to the vials, and samples were stored at 4°C until processing, which was carried out several days after the cruise. After the addition of scintillation cocktail (ULTIMA-GOLD), the samples were counted using a TRI-CARB 2100 TR (PACKARD) scintillation counter. Bacterial productivity (BP) and BP/PP ratio measurements were carried out for the same depths as those for N$_2$ fixation and PP (4–6 m depths at the upper 150 m).

3. Results

[15] The water column was characterized by typical summer oligotrophic conditions, with a well-stratified mixed surface layer (~20 m). Water temperatures ranged from 29°C at the surface layer to ~17°C at the bottom of the photic layer (~150 m). Salinity ranged between 39.8 and 38.7 for the surface and 150 m depth, respectively. The cyclonic Rhodes Gyre was also stratified, with cool (~23°C) and less saline (~39) waters ascending up to 40 m, while temperature and salinity reached the background levels (i.e., >26°C and 39.4, respectively) at the surface layer (5 m). The anticyclonic Cyprus Eddy was characterized by a warm and saline top layer (~27°C and 39.4, respectively). Below the thermocline (~30 m), temperature decreased to ~17°C, while salinity remained unchanged at ~39.3 (Table 1).

[16] The depth-integrated (top 150 m) NO$_2$ + NO$_3$ and PO$_4$ concentrations throughout the Levantine basin ranged from 15 to 53 mM m^{-2} and from 1.5 to 5.8 mM m^{-2} for N and

P, respectively, with the higher concentrations found at the more western stations of the Levantine basin. Within the Rhodes Gyre, high areal NO$_2$ + NO$_3$ concentrations were observed (136 mM m^{-2}), while sixfold lower values were measured (26 mM m^{-2}) within the Cyprus Eddy. No significant differences between the gyre and the eddy were observed for the PO$_4$ integrated values (~3 mM m^{-2}) (Table 1).

[17] Measured PP rates across the Levantine basin revealed an east-west gradient ($R^2 = 0.71$, $n = 11$, $P < 0.001$) (Figure 2a). The PP values at the easternmost stations were lower by a factor of 10 compared to those at the western Levantine basin stations (~200 and >2000 μmol C m^{-2} d^{-1}, respectively) (Figure 2a). Areal PP rates within the Cyprus Eddy and Rhodes Gyre were 2150 and 2300 μmol C m^{-2} d^{-1}, respectively (Figure 2a), regardless of the physical characteristics of the gyre or eddy.

[18] Areal N$_2$ fixation rates were low throughout all sampled stations in the Levantine basin. In contrast to the PP rates (Figure 2a), no clear east-west gradient was observed for the N$_2$ fixation rates (Figure 2b). N$_2$ fixation rates ranged from 0.8 μmol N m^{-2} d^{-1} at the station closest to the coast to 3.2 μmol N m^{-2} d^{-1} at Station IL16. The highest areal N$_2$ fixation rates were within the Rhodes Gyre and Cyprus Eddy (19.5 and 22.5 μmol N m^{-2} d^{-1}, respectively), higher by 1 order of magnitude than those in all other eastern Mediterranean Sea stations uninfluenced by gyre or eddy circulations (Figure 2b).

[19] The percent contribution of the biologically fixed N to PP at the background stations (ranging from 0.7% to 2%, $n = 96$) was threefold to sixfold lower than that in both gyres (>6%, $n = 24$) (Figure 2c). In the eastern Levantine stations, we calculated a higher contribution of the fixed N to PP (2%) than in the more western stations (0.7%), yet these did not differ significantly (t test, $P > 0.05$) (Figure 2c).

[20] Our results show that during the stratified period, excluding the Rhodes Gyre and Cyprus Eddy, N$_2$ fixation was uncoupled from PP across the Levantine basin. Other studies from varying oceanic regions demonstrated positive correlation of PP with N$_2$ fixation in the photic illuminated layer under stratified conditions (Figure 3, $R^2 = 0.54$, $P = 2 \times 10^{-6}$, $n = 26$). Our results contrast with these studies. We did not find a significant correlation in the Levantine basin ($P = 0.3$, $n = 19$) (Figure 3). Moreover, differential responses

between N$_2$ fixation, PP, and bacterial production resulted when surface seawater (5 m) was artificially enriched with 100 nM PO$_4^{-3}$ (Figure 4) to examine the P limitation of dia-
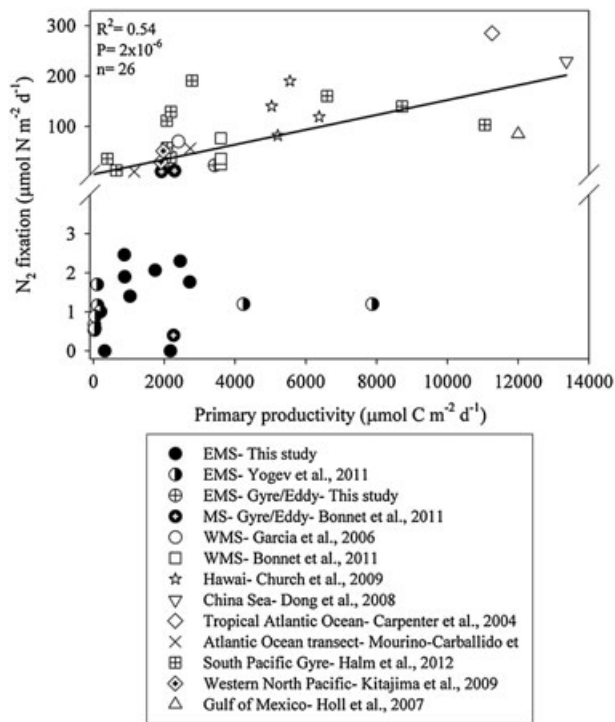

Figure 3. Correlation between N$_2$ fixation rates and PP for the illuminated photic depths (depending on the study area, this depth ranged from 100 to 150 m). Data were compiled from this study, the eastern Mediterranean Sea (EMS) [*Yogev et al.*, 2011], anti-cyclonic eddies within the Mediterranean Sea [*Bonnet et al.*, 2011], the DYMAMED station in the WMS [*Garcia et al.*, 2006], Station ALOHA near Hawaii [*Church et al.*, 2009], the South China Sea [*Dong et al.*, 2008], the tropical and subtropical western North Pacific [*Kitajima et al.*, 2009], the Gulf of Mexico [*Holl et al.*, 2007], the Atlantic Ocean [*Mourino-Carballido et al.*, 2011], the Baltic Sea [*Ohlendieck et al.*, 2007], and the South Pacific Gyre [*Halm et al.*, 2012].
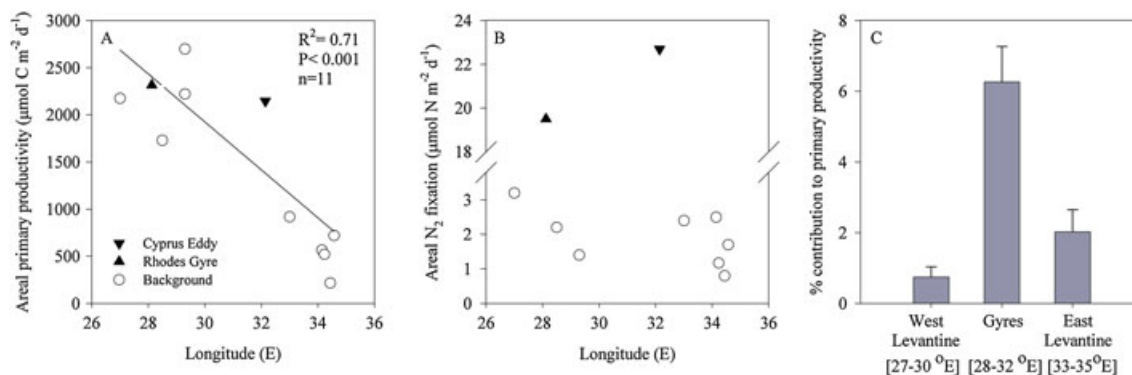

Figure 2. Areal (0–150 m) PP (a), N$_2$ fixation (b), and percent contribution of N$_2$ fixation to PP in the Levantine basin (c) during the stratified summer months from the different water provinces of the Levantine basin. White symbols represent the uninfluenced Levantine basin stations, while black triangles represent the Cyprus Eddy and Rhodes Gyre.
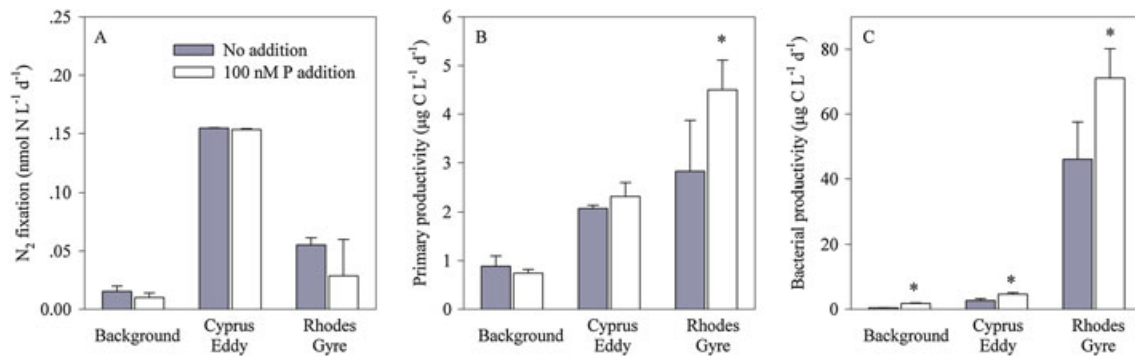
Figure 4. The influence of P enrichment on N$_2$ fixation rates (a), PP (b), and BP (c) from surface waters (5–20 m) of representative stations of Levantine pelagic water (Station 10), the Cyprus Eddy, and the Rhodes Gyre during July 2009. The gray bars represent the control treatments (no addition), while the white bars represent the rates measured after incubation for 24–30 h with 100 nM PO$_4$ addition. Values are averages and error bars are standard deviations of three independent replicates performed for each control and treatment incubation. The asterisks above the columns represent statistically significant differences (one-way ANOVA and a Fisher LSD means comparison test, $P < 0.05$) for mean values of P additions between stations.

zotrophy. PO$_4^{-3}$ additions did not stimulate N$_2$ fixation at any of the selected stations (Figure 4a). PP was enhanced significantly only at the Rhodes Gyre (Figure 4b), while bacterial production increased significantly at all experimental stations (Figure 4c). A 4-fold increase was observed in BP rates in the uninfluenced station (i.e., background), while an ~1.5-fold increase was measured within the gyre and eddy (Figure 4c).

4. Discussion

[21] Our data substantiate previous studies reporting typically low rates of N$_2$ fixation for the Mediterranean Sea, specifically the eastern Mediterranean Sea [*Ibello et al.*, 2010; *Yogev et al.*, 2011; *Bonnet et al.*, 2011; *Ridame et al.*, 2011; *Berman-Frank and Rahav*, 2012]. Moreover, the results of this study distinctly demonstrate that summertime N$_2$ fixation within the photic zone of the Mediterranean Levantine basin provides a minor contribution to total production (Figure 2c) and is uncoupled from primary production, excluding isolated "hot spots" such as the Rhodes Gyre or Cyprus Eddy (Figure 3).

[22] Why are N$_2$ fixation and PP uncoupled in the eastern Mediterranean Sea? This question warrants special attention especially as our results contrast with the significant and positive correlation found between these processes from several oceanic ecosystems as well as the more productive western Mediterranean Sea (WMS) ($R^2 = 0.54$, $P = 2 \times 10^{-6}$, $n = 26$) (Figure 3). High concurrent rates of N$_2$ fixation and PP were associated with the dominance of species of the diazotrophic cyanobacterium *Trichodesmium* in the South China Sea [*Dong et al.*, 2008] and the Atlantic Ocean [*Fernandez et al.*, 2010; *Mourino-Carballido et al.*, 2011]. The contribution of *Trichodesmium* to PP was 11.6% in the outer Sanya Bay of the China Sea [*Dong et al.*, 2008] and as high as 22%–44% in the equatorial Atlantic Gyre and South Atlantic Gyre [*Fernandez et al.*, 2010; *Mourino-Carballido et al.*, 2011]. Furthermore, *Trichodesmium* accounted on average for about 47% of total depth-integrated primary production in the North Atlantic Ocean [*Carpenter et al.*,

2004]. The positive correlation between PP and N$_2$ fixation demonstrated in Figure 3 is not limited to *Trichodesmium* and filamentous cyanobacteria comprising the diazotrophic community. At Station ALOHA (Hawaii Ocean Time-series program in the central North Pacific Subtropical Gyre), the diazotrophic community was composed mainly of unicellular organisms in the late winter and early spring [*Church et al.*, 2009], while in the South Pacific Gyre, gamma proteobacteria and unicellular photoheterotrophic Group A prevailed [*Halm et al.*, 2012]. Other studies demonstrated that diazotrophy from both *Trichodesmium* and diatom-cyanobacterial diazotrophic associations positively correlated with increased C drawdown [*Subramaniam et al.*, 2008]. In the WMS and the central Ligurian Sea (NW Mediterranean Sea), coupling between N$_2$ fixation and PP was positive, excluding several measurements from DYFAMED during the oligotrophic stratified summer, when high N$_2$ fixation rates were associated with low PP rates [*Garcia et al.*, 2006; *Bonnet et al.*, 2011]. Similarly, within the Cyprus Eddy and Rhodes Gyre (this study), N$_2$ fixation and PP measurements corresponded with the linear regression produced for the more productive environments described above (Figure 3).

[23] Presently, published data including both N$_2$ fixation and PP are limited, so we could not verify whether the uncoupling shown between N$_2$ fixation and PP from the eastern Mediterranean Sea data corresponds to other areas of low, photic zone, N$_2$ fixation (<10–20 μmol N m^{-2} d^{-1}) (Figure 3). We believe that this uncoupling will be found in other ultraoligotrophic environments and should be further investigated.

[24] These examples illustrate the general coupling found between N$_2$ fixation rates (and diazotrophic organisms) and PP in the photic layer and contrast with our findings for the eastern Mediterranean Sea demonstrating uncoupling between these two processes ($n = 19$, $P = 0.3$) (Figure 3). This uncoupling in the eastern Mediterranean Sea may be explained by examining the characteristic composition and structure of the pelagic primary producers and the resulting microbial food web. In the ultraoligotrophic eastern Mediterranean basin, primary production is attributed predominantly to the

picophytoplankton—mainly picoeukaryotes, nanoeukaryotes, prochlorophytes, and *Synechococcus* types [*Yacobi et al.*, 1995; *Psarra et al.*, 2000; *Tanaka et al.*, 2007; *Ignatiades et al.*, 2009; *Denis et al.*, 2010]. Microphytoplankton blooms occur only occasionally [*Ignatiades et al.*, 2009], with higher biomass blooms limited mostly to coastlines or gyres [*Siokou-Frangou et al.*, 2010] and diazotroph blooms being a rare event [*Spatharis et al.*, 2012]. Recent genomic characterization of eastern Mediterranean Sea surface waters yielded predominant sequences corresponding to Alphaproteobacteria (SAR11-like and Rhodobacterales), Cyanobacteria (*Synechococcus* and high light–adapted *Prochlorococcus*), and diverse uncultured Gammaproteobacteria [*Feingersch et al.*, 2009]. Furthermore, the extremely limited concentrations of dissolved inorganic nitrogen and phosphorus cause interspecific competition between phytoplankton and heterotrophic bacteria for nutrients and induce efficient biological recycling of dissolved inorganic and organic nutrient pools [*Pitta et al.*, 2005; *Lagaria et al.*, 2010]. Thus, genomic analysis of the eastern Mediterranean Sea demonstrated an enrichment of sequences related to phosphate and/or phosphonate uptake and assimilation [*Feingersch et al.*, 2009]. Rapid uptake and utilization of P sources by heterotrophic bacteria can lead to systems with elevated BP and a decline in PP as measured in a large-scale P fertilization experiment in the eastern Mediterranean Sea (CYCLOPS) [*Pitta et al.*, 2005; *Thingstad et al.*, 2005].

[25] The BP/PP ratio is not diazotroph specific yet provides a measure of the metabolic status of the environment, i.e., whether the environment is predominantly heterotrophic or autotrophic (similar to the bacterial carbon demand to PP ratio described by *Lagaria et al.* [2010]). A ratio <1 indicates higher autotrophic fixation of carbon relative to heterotrophic carbon fixation; conversely, when this ratio is >1, there is higher heterotrophic production. In this study, the BP/PP ratio was <1 for the pelagic background stations uninfluenced by vortexes (0.2–0.5), demonstrating autotrophic metabolism; ~1 at the Cyprus Eddy; and >1 at the Rhodes Gyre (~12), indicating heterotrophic metabolism.

[26] The metabolic status suggestive of heterotrophic metabolism in the Rhodes Gyre and Cyprus Eddy corresponds with the highest areal N$_2$ fixation rates we measured during the summer stratification (19–23 μmol N m^{-2} d^{-1}), while the lowest N$_2$ fixation was obtained from the Levantine background stations (~2 μmol N m^{-2} d^{-1}) (Figure 2b), corresponding with previous reports for the eastern Mediterranean Sea [*Ibello et al.*, 2010; *Bonnet et al.*, 2011; *Yogev et al.*, 2011]. Moreover, the maximum volumetric rates of N$_2$ fixation (>0.3 nmol N L^{-1} d^{-1}) were measured within the Rhodes Gyre at aphotic depths >130 m, suggesting a greater contribution by heterotrophic diazotrophs from the aphotic layer.

[27] Although we presently do not have a molecular fingerprint of the diazotroph communities at our stations, we did not observe any conspicuous populations of cyanobacterial or diatom-cyanobacterial diazotrophic associations throughout our samplings in the upper photic layers of the eastern Mediterranean Sea over the past 7 years. While phototrophic (cyanobacterial) diazotrophs enjoy a direct (solar) energy source for the energetically expensive process of N$_2$ fixation, the presence and contribution of non-cyanobacterial heterotrophic diazotrophs in marine systems are being increasingly recognized [*Zehr and Turner*, 2001; *Riemann et al.*, 2010; *Fernandez et al.*, 2011]. Some of these organisms have also been found in the eastern Mediterranean Sea close to our current stations [*Man-Aharonovich et al.*, 2007; *Ibello et al.*, 2010; *Yogev et al.*, 2011]. During the BOUM campaign (June 2008), the diazotrophic populations at Station C (located within the Cyprus Eddy) were predominantly <3 μm and composed of such bacteria as *Bradyrhizobium*, *Rhizobium*, and Gammaproteobacteria [*Bonnet et al.*, 2011; *Le Moal et al.*, 2011; *Ridame et al.*, 2011]. The N$_2$ fixation rates of these populations in the Cyprus Eddy (subsurface Station C waters from 8 m: 0.09 nmol N L^{-1} d^{-1} [*Bonnet et al.*, 2011; *Ridame et al.*, 2011]) are similar to our rates measured 1 year later: ~0.15 nmol N L^{-1} d^{-1} from surface to 150 m.

[28] To investigate the parameters controlling the variability in N$_2$ fixation, we examined the chemical and physical characteristics that would enable 1 order of magnitude higher N$_2$ fixation rates in the gyre and eddy compared to the surrounding Levantine waters. Phosphorus, essential for the energetically expensive process of N$_2$ fixation [*Postgate*, 1979], has previously been considered limiting for diazotrophy in the eastern Mediterranean Sea [*Rees et al.*, 2006; *Krom et al.*, 2010]. Our midsummer transect of the eastern Mediterranean Sea occurred when surface waters were depleted in dissolved inorganic phosphate concentrations that were below or close to their analytical detection limits and were also similar for the upper 150 m between the Rhodes Gyre and the Cyprus Eddy. PO$_4$ additions to surface seawater samples did not stimulate diazotrophy at any of the stations (Figure 4a). We conclude that during the ISRLEV cruise (July 2009; see Table 1), dissolved inorganic phosphorus was not the limiting factor for N$_2$ fixation, in contrast to a June 2008 transect (BOUM) where similar concentrations of PO$_4$ additions to the Cyprus Eddy waters increased N$_2$ fixation rates by threefold over the unamended controls [*Ridame et al.*, 2011]. Co-limitation of diazotrophs by other microelements could occur as exhibited by addition of Saharan dust (containing elements such as Fe, Mo, and Zn) to seawater that enhanced N$_2$ fixation in surface waters fivefold in the Cyprus Eddy relative to the surrounding water mass [*Ridame et al.*, 2011]. However, we did not examine this issue here and suggest further experimentation is required to understand what controls both autotrophic diazotrophy and heterotrophic diazotrophy in the eastern Mediterranean Sea.

[29] During our transects (summer stratified period), the fixed nitrogen (NO$_2$ + NO$_3$) concentrations in the Rhodes Gyre were sixfold higher than those measured in the Cyprus Eddy (Table 1), while PP rates at the gyre and eddy were similar (Figure 2a). These indicate that the increase in fixed N sources in the Rhodes Gyre was not sufficient to sustain higher PP with the available concentrations of dissolved inorganic phosphorus and thus allude to PP, which is P limited as typically described for the eastern Mediterranean Sea [*Krom et al.*, 1991; *Zohary and Robarts*, 1998; *Van-Wambeke et al.*, 2002; *Tanaka et al.*, 2009]. Indeed, we confirmed that during our sampling, P availability limited phytoplankton in the Rhodes Gyre, as PP increased when surface water bioassays were enriched with PO$_4$ (Figure 4b). In the Cyprus Eddy, PP did not increase in PO$_4$-amended treatments (Figure 4b) and may thus be N and P co-limited as described in the BOUM [*Ridame et al.*, 2011; *Tanaka et al.*, 2011] and CYCLOPS [*Zohary et al.*, 2005; *Tanaka*

et al., 2007] campaigns. The substantial increase in bacterial production when PO$_4$ was added (1.5-fold to 4-fold), compared to unamended controls (Figure 4c), highlights the competitive ability of heterotrophic bacteria to efficiently take up and utilize inorganic P as previously observed in the eastern Mediterranean Sea [*Thingstad et al.*, 2005]. Furthermore, non-diazotrophic heterotrophic bacteria may in fact outcompete diazotrophs if another resource (e.g., dissolved Fe) is limiting. These interactions place an additional control on photosynthetic autotrophs and diazotrophic biomass.

[30] In conclusion, our data demonstrate that N$_2$ fixation in the photic zone of the eastern Mediterranean Sea contributes only negligibly by direct inputs to PP (i.e., cyanobacterial diazotrophs) and is in fact uncoupled from PP. In contrast, N$_2$ fixation is significantly coupled to BP and high N$_2$ fixation coincides with net heterotrophic areas. We suggest that in this system, where heterotrophic bacteria compete with the primary producers for the limited nutrients [*Pitta et al.*, 2005; *Thingstad et al.*, 2005; *Lagaria et al.*, 2010], heterotrophic N$_2$ fixation can be significant and may also extend deep into the aphotic depths.

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