



RESEARCH LETTER

10.1029/2018GL077132

Key Points:

- Aerosols may contain diverse and viable diazotrophs and other prokaryotes
- Aerosol-associated diazotrophs can fix N₂ in the NRS
- Dust storms contribute significantly to N₂ fixation in NRS

Supporting Information:

- Supporting Information S1

Correspondence to:

E. Rahav,
eyal.rahav@ocean.org.il

Citation:

Rahav, E., Paytan, A., Mescioglu, E., Galletti, Y., Rosenfeld, S., Raveh, O., et al. (2018). Airborne microbes contribute to N₂ fixation in surface water of the Northern Red Sea. *Geophysical Research Letters*, 45. <https://doi.org/10.1029/2018GL077132>

Received 18 JAN 2018

Accepted 4 JUN 2018

Accepted article online 12 JUN 2018

Airborne Microbes Contribute to N₂ Fixation in Surface Water of the Northern Red Sea

Eyal Rahav¹, Adina Paytan², Esra Mescioglu², Yuri Galletti³, Sahar Rosenfeld¹, Ofrat Raveh¹, Chiara Santinelli³, Tung-Yuan Ho⁴, and Barak Herut¹

¹Israel Oceanographic and Limnological Research, National Institute of Oceanography, Haifa, Israel, ²Institute of Marine Science, University of California, Santa Cruz, CA, USA, ³Institute of Biophysics, C.N.R, Pisa, Italy, ⁴Research Center for Environmental Changes, Academia Sinica, Taipei, Taiwan

Abstract Desert dust storms are frequent in the Northern Red Sea region, providing nutrients (i.e., PO₄) and trace-metals (i.e., Fe) that may stimulate dinitrogen (N₂) fixation. Dust also carries a high diversity of airborne microbes (bacteria and archaea), including diazotrophs, that may remain viable during transport and upon deposition. Here we evaluate the impact of atmospheric deposition and its associated airborne diazotrophs on N₂ fixation in the surface water of the low-nutrient Northern Red Sea, using mesocosm bioassay experiments. We compared the chemical (nutritional) and sole airborne microbial impact of aerosol additions on N₂ fixation using “live-dust” (release nutrients/trace metals and viable airborne microorganisms) and “UV-killed dust” (release only chemicals). Airborne diazotrophy accounted for about one third of the measured N₂ fixation (0.35 ± 0.06 nmol N · L⁻¹ · day⁻¹ and 0.29 ± 0.06 nmol N · L⁻¹ · day⁻¹, for “February 2017” and “May 2017,” “live-dust” additions, respectively). Two *nifH* sequences related to cluster III diazotrophs were amplified from the dust samples, consistent with the N₂ fixation measurement results. We postulate that the deposition of viable airborne diazotrophs may enhance N₂ fixation, especially in marine provinces subjected to high aerosol loads. We speculate that the relative contribution of airborne N₂ fixation may increase in the future with the predicted increase in dust deposition.

Plain Language Summary Aerosols and dust are regularly transported across the oceans supplying nutrients and trace metals to the surface water. In addition, aerosols may also contain a wide array of different airborne microorganisms (heterotrophic bacteria, virus, cyanobacteria, and fungi) that can be easily transported for thousands of kilometers away from their place of origin within a few days. Here we examined the role of airborne N₂ fixers (diazotrophs) in the surface water of the low-nutrient Northern Red Sea during the summer. To this end, we compared the chemical (nutritional) and sole airborne microbial impact of aerosol additions on N₂ fixation using “live-dust” (release nutrients/trace metals and viable airborne microorganisms) and “UV-killed dust” (release only chemicals). Our results demonstrate that airborne N₂ fixation may be an important source of new bioavailable N in the Northern Red Sea, fueling primary production. In accordance with these measurements, two *nifH* sequences related to cluster III diazotrophs were amplified from the dust samples. Our results suggest that airborne N₂ fixation may play an important role in marine environments subjected to high aerosol loads. We further suggest that the role of airborne diazotrophs may likely increase in the future due to global desertification processes and thus an increase in dust deposition.

1. Introduction

Dinitrogen (N₂) fixation plays a pivotal role in the marine nitrogen (N) cycle by providing bioavailable N to phytoplankton and prokaryotes (Gruber & Galloway, 2008; Sohm et al., 2011). Many factors can affect N₂ fixation rates, including the availability of P (Dyhrman & Haley, 2006; Sanudo-Wilhelmy et al., 2001), Fe (Berman-Frank et al., 2007; Moore et al., 2009), dissolved organic carbon (DOC) compounds such as glucose (Rahav & Bar-Zeev, 2017; Rahav, Giannetto, & Bar-Zeev, 2016), amino-acids (Rahav et al., 2015), as well as physical conditions in the water column like temperature (Capone et al., 2005) and light (Berman-Frank et al., 2001).

The release of nutrients from aerosols deposited into the ocean can elevate N₂ fixation rates (Foster et al., 2009; Gruber & Sarmiento, 1997; Herut et al., 2016; Marañón et al., 2010; Ridame et al., 2013) and alter the diversity of the diazotrophic community (Rahav, Shun-Yan, et al., 2016). Aerosols can also transport viable airborne diazotrophs, which are able to fix N₂ upon addition to sterile seawater (Rahav, Ovadia, et al., 2016). This is consistent with the identification of *nifH* genes in aerosol samples collected at the Northern Red Sea (NRS;

Foster et al., 2009). Therefore, elevated N_2 fixation rates, measured following aerosol additions to the ocean surface, may be attributed to both the addition of chemical constituents supplied by the aerosols (i.e., P, DOC, and Fe), as well as to the addition of viable airborne diazotrophs (Rahav, Ovadia, et al., 2016).

We examine here the contribution of airborne diazotrophy to overall N_2 fixation in the NRS, a basin surrounded by deserts and hence receives relatively high aerosol deposition throughout the year (Banks et al., 2017; Chen et al., 2008; Jish Prakash et al., 2015; Torfstein et al., 2017). Further, the NRS is highly oligotrophic and stratified during summertime (Dishon et al., 2012; Meeder et al., 2012; Rahav et al., 2015); thus, external nutrients and airborne microorganisms may contribute a proportionally high fraction of active microbes to the top layer standing stock (Rahav, Shun-Yan, et al., 2016). Lastly, previous studies at the NRS reported that P availability may at times limit N_2 fixation (Foster et al., 2009; Rahav et al., 2015). Therefore, P addition from atmospheric aerosols may enhance N_2 fixation rates.

In this study, we used mesocosm bioassay experiments to evaluate the impact of bulk desert dust atmospheric deposition, and particularly the impact of airborne diazotrophs in the dust, on N_2 fixation rates in the NRS during summer. To the best of our knowledge, this is the first attempt to evaluate the role of airborne diazotrophy under natural conditions rather than in sterile laboratory-controlled settings (Rahav, Ovadia, et al., 2016). We show that airborne diazotrophs contribute to N_2 fixation in the NRS.

2. Methods

2.1. Mineral Dust Collection

Dust was collected during two storm events (28 February 2017 and 18 May 2017) using glass plates at the Interuniversity Institute, Eilat, Israel (29°28'N, 34°55'E) and placed in sterile Eppendorf tubes (a few grams from each event were collected). Total dust particles were collected, that is, without any sieving to remove large (local) particles. Dust samples were kept frozen until used in this experiment. Prior to the experiment, the samples were homogenized by vortex and transferred to new sterile and clean Eppendorf tubes (each contained 240–250 mg). Triplicate dust subsamples from each dust type were then put under a UV light for 48 h (“UV-killed dust”) in a biological hood (SLEE, London). Dust samples (“live-dust” or UV-killed dust) were added to the different mesocosms and were washed three times with ambient seawater to make sure all particles were removed and added to the mesocosms. Three-day back trajectories arriving at 100, 500, and 1,000 m altitude were calculated for the two dust samples (Figure 1).

2.2. Mesocosm Setup and Experimental Design

Waters were collected from the surface mixed layer (~10 m depth) in the NRS (Gulf of Aqaba) on 9 July 2017 by pumping. The collected seawater was distributed homogeneously between 15 costume-made precleaned (10% HCl) polycarbonate mesocosms bags ($r = 0.3$ m, $h = 1.5$ m, 300 L added seawater). The mesocosms were deployed in a circulating seawater pool to maintain the ambient temperatures (25–28 °C) and covered with a shading net to maintain ambient light (80–100 $\mu\text{mol quanta} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ during midday, LI-COR PAR sensor). The mesocosms were mixed manually every 6–8 hr to assure uniform distribution of the dust particles. No thermal stratification was recorded in the pool or the mesocosm bags during the experiment duration. The following amendments were made in triplicates: (1) no addition (control), (2) untreated live-dust collected in February 2017, (3) UV-killed dust collected in February 2017, (4) untreated live-dust collected in May 2017, and (5) UV-killed dust collected in May 2017. The live-dust contains nutrients, trace metals, and viable airborne microorganisms that may be release from particles upon deposition in the surface seawater. Adversely, the UV-killed dust releases to the water only nutrients and trace metals, while the airborne microbes (not only diazotrophs) were not active due to the damaging effects of UV exposure. Indeed, the UV pretreatment removed >95% of all airborne bacteria (Figure S1). The difference in response between the treatments can be attributed primarily to the effects of the “live” microbes in the aerosol samples (Rahav, Ovadia, et al., 2016; Rahav, Paytan, et al., 2016).

The amount of aerosol added to each mesocosm was ~0.8 mg/L, which is within the range of natural deposition to the upper mixed layer of the NRS (~15 m) during intense dust storm events (Chen et al., 2008; Jish Prakash et al., 2015). The mesocosms were sampled for a suite of variables at 0, 6, 48, and 72 hr post addition during morning time (08:30 local time) using acid-clean Tygon tubing from ~0.5 m depth. Waters were extracted via suction and gravity flow as described in Herut et al. (2016) after shaking for homogenization.

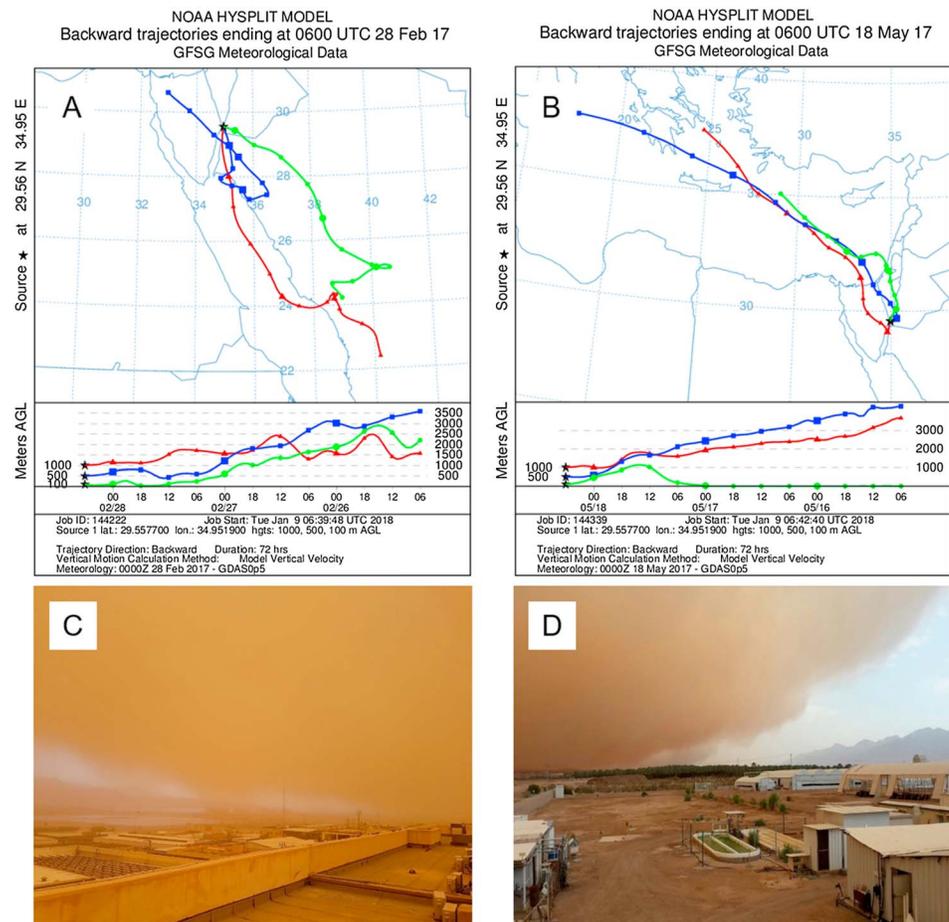


Figure 1. The origin of the aerosols used in the mesocosm experiment based on three-day back trajectories (100, 500, and 1,000 m altitude). Aerosols were collected at the study site (Northern Red Sea) in (a and c) February 2017 and (b and d) May 2017 during dust storms.

The collected seawater was transferred to the sampling bottles and taken to the lab within a few minutes for further analyses as described below.

2.3. DNA Extraction and *nifH* Gene Diversity

DNA was extracted from the aerosol samples using the phenol-chloroform method according to Man-Aharonovich et al. (2007). Nitrogenase Fe protein transcripts (*nifH*) were amplified with a nested polymerase chain reaction strategy (Zehr & McReynolds, 1989). More details are provided in the supporting information.

2.4. Nutrients and Trace Metals Release From the Aerosols

A week prior to the mesocosm experiment, ~ 25 mg of the collected aerosol samples were added to 1 L of pre-filtered (0.2 μm), aged, and poisoned (HgCl_2) seawater and shaken in the dark at room temperature for 60 hr (Herut et al., 2002). Triplicate subsamples were syringe-filtered through a 0.2 μm filter and immediately analyzed (without a freezing step) for $\text{NO}_3 + \text{NO}_2$ and PO_4 by a photometric segmented flow method (Seal Analytical AA-3 system, Herut et al., 1999). The precision for $\text{NO}_3 + \text{NO}_2$ and PO_4 was 20 and 3 nM, respectively.

Trace metals in the solution were separated from the salt matrix, preconcentrated, and measured using a sector field high-resolution inductively coupled plasma-mass spectrometry (Element XR, Thermo, USA) with an autosampler (ESI, SC-4, USA) coupled with a PEEK standard probe (Ho et al., 2010; Wang et al., 2014). More details are given in the supporting information.

2.5. DOC

Samples for DOC analysis (200 ml) were immediately filtered through a prewashed (500 ml of Milli-Q water) 0.2 μm Nylon filter and stored at 4 $^\circ\text{C}$ until the analysis (approximately one month). Measurements were

carried out with a Shimadzu TOC-Vcsn (Santinelli et al., 2013). Values were compared with Consensus Reference Waters (CRM; batch 15 Lot #07-15). More details are provided in the supporting information.

2.6. Bacterial Abundance

Seawater samples (1 ml) were fixed with 50% glutaraldehyde (Sigma G7651), stained with 0.5 nM SYTO9 (Applied-Biosystems) and analyzed with an Attune acoustic focusing flow-cytometer (Applied-Biosystems) as described in Rahav and Bar-Zeev (2017).

2.7. Bacterial Production

Rates of bacterial production were estimated using the [4,5-³H]-leucine incorporation method (Simon et al., 1990). More details are provided in the supporting information.

2.8. Dinitrogen (N₂) Fixation

¹⁵N₂-enriched seawater (Mohr et al., 2010) was added to 4.6 L Nalgene bottles containing the different mesocosm treatments (5% of the total volume). A recent study from the NRS showed that such addition yields the highest N₂ fixation rates without overdiluting the sample with ¹⁵N₂-enriched seawater (Rahav et al., 2015). The bottles were incubated in the same pool where the mesocosms were placed for 24 hr and filtered through precombusted (450 °C, 4.5 hr) GF/F filters (Whatman). The filters were dried in an oven at 60 °C overnight and analyzed using a CE Instruments NC2500 elemental analyzer interfaced to a Thermo-Finnigan Delta Plus XP isotope ratio mass spectrometer.

2.9. Beta-Glucosidase Activity (β-Glu)

The activity of β-glucosidase was determined by the 4-methylumbelliferyl-β-D-glucopyranoside method as described in Hoppe (1983). β-Glucosidase is an ecto-enzyme that enables prokaryotes (including heterotrophic and autotrophic diazotrophs) to hydrolyze glucose from large organic molecules that are otherwise unavailable for the cells (Hoppe, 1983). In turn, this allows rapid utilization of the free-bioavailable DOC source by diazotrophs to mediate/sustain N₂ fixation (Rahav, Giannetto, et al., 2016). More details are given in the supporting information.

2.10. Statistic Analysis

Data are displayed as averages and standard deviations ($n = 3$). A repeated measure analysis of variance (ANOVA) followed by Tukey's posthoc test was applied ($P < 0.05$) to compare the differences between the control and the dust addition treatments (two types, and live versus "killed" treatments). Prior to analyses, the normality and heterogeneity of the variances of the data were examined. The data presented in Figure 2 show the mean \pm standard-deviation from three independent replicate mesocosms. The relationship between the N₂ fixation and bacterial abundance, bacterial production, and β-glu variables was determined with a Pearson correlation test ($P < 0.05$). The results of both aerosol treatments ("February 2017" and "May 2017") and sampling days were pooled for this relationship analysis ($n = 8$). All tests were performed using the Excel add-on package XLSTAT.

3. Results and Discussion

Two types of aerosols dominated by mineral dust were used, representing different origins and atmospheric transport routes prior to deposition at the study site (Figure 1). Air-mass back trajectory shows that the February 2017 sample was more local and originated from south-east (an "eastern Mediterranean" source), while the May 2017 sample started from a northern European source. Correspondingly, the bioavailable nutrient (NO₃ + NO₂, PO₄, and DOC) and trace element characteristics exhibited distinct chemical differences (Table 1). In the February 2017 sample, each milligram of aerosol contributed ~20 nmol NO₃ + NO₂ and ~2 nmol PO₄ to seawater, resulting in N:P ratio of 10:1 (mol:mol). Higher nutrient concentrations were released from the May 2017 particles with ~60 nmol N and ~3 nmol P per mg of dust, resulting in N:P of ~20:1 (mol:mol). Thus, the addition of 0.8 mg/L of aerosol to surface seawater resulted in the addition of ~48 nM NO₃ + NO₂ (+34%) and ~2.4 nM PO₄ (+30%) for the May 2017 mesocosm, and ~16 nM NO₃ + NO₂ (+11%) and 1.6 nM PO₄ (+20%) for the February 2017 mesocosm (Tables 1 and 2). Addition of PO₄ was previously shown to stimulate N₂ fixation in the NRS (Foster et al., 2009; Rahav et al., 2015), while the low NO₃ + NO₂ concentrations in the water column (Table 2) and the relatively low N:P ratios in these specific aerosols (e.g., 10–20 compared to an average of

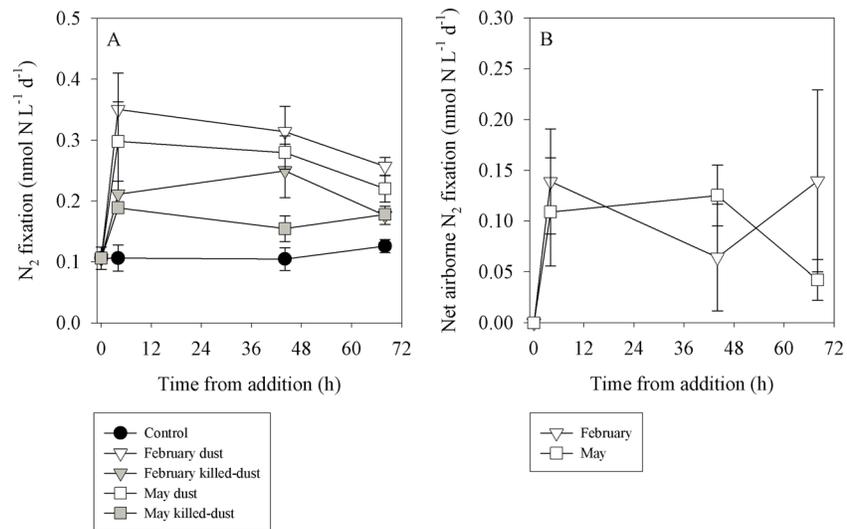


Figure 2. Temporal changes in volumetric (a) N_2 fixation rates and the (b) net airborne diazotrophic activity following dust additions (~ 0.8 mg/L) to surface seawater of the Northern Red Sea during July 2017. The following treatments were performed: dust collected in February 2017 (white triangle), UV-killed February 2017 dust (gray triangle), dust collected in May 2017 (white square), UV-killed May 2017 dust (gray square), and unamended controls (black circle). Data shown are the averages of three replicate mesocosms per treatment and their corresponding standard deviation. Note the different Y axis. The net airborne diazotrophic contribution was calculated by subtracting the “killed-dust” rates from the rates measured in the “live-dust” treatments.

190 during nonstorm events; Chen et al., 2008) are not likely to inhibit this process (Knapp, 2012). The leached DOC concentrations also differed between aerosol types, with 64 ± 3 nM released from the February 2017 aerosol and 125 ± 2 nM from the May 2017 particles (Table 1). These DOC values were similar to the ambient concentrations measured at the beginning of the experiment, resulting in a net increase of 86–169% (Table 2). Trace metals leached to seawater from the two aerosol samples are presented in Table 1. Amendment of 0.8 mg/L aerosols resulted in addition of <3.3 nM Fe (up to +39%), ~ 7 –15 nM Zn (+77–179%), and <1 nM Cu (+28–42%; Table 1). These concentrations were additive to the ambient concentrations measured in the NRS water (Table 2, (Chase et al., 2006, 2011)). Any change in N_2 fixation rates following aerosol addition (February 2017 or May 2017) may be explained by the leached micronutrients and macronutrients that relieve diazotrophy-limiting constituents (i.e., PO_4 ; Foster et al., 2009; Rahav et al., 2015; DOC, Benavides et al., 2018; Rahav, Giannetto, et al., 2016; and/or trace elements; Mills et al., 2004; Moore et al., 2009), as well as by a contribution from airborne diazotrophs (Figure 2 and see discussion below).

N_2 fixation rates in the water prior to adding the dust were overall low (0.11 ± 0.02 nmol $N \cdot L^{-1} \cdot day^{-1}$; Table 2) and exhibited similar values to those previously reported for the NRS (Foster et al., 2009; Rahav et al., 2015). The

Table 1
Leached Nutrients and Trace Metals From the Aerosols Collected in February and May 2017 and Calculated Net Increase of Ambient Concentration in the Mesocosms Following ~ 0.8 mg/L of Dust Addition

Variable	Leached element conc. (nmol/mg dust)		Concentration in the mesocosm (nM)	
	February 2017	May 2017	February 2017	May 2017
$NO_3 + NO_2$	20 ± 3	60 ± 4	16 ± 2	48 ± 2
PO_4	2 ± 1	3 ± 1	<2	2.4 ± 1
DOC ^a	81 ± 4	157 ± 7	64 ± 3	125 ± 2
Fe	BDL	3.3 ± 1.9	BDL	2.6 ± 1.5
Zn	19.5 ± 3.2	8.4 ± 3.1	15.6 ± 2.5	6.7 ± 2.5
Cu	0.5 ± 0.1	0.7 ± 0.2	0.4 ± 0.1	0.6 ± 0.2

Note. Data shown are average \pm standard deviation from three independent replicates. BDL, below detection limit.

Table 2
Chemical and Biological Properties of the NRS Surface Water During July 2017 Before Amendments Were Made

Parameter	Unit	Average \pm SD ($n = 3$)
NO ₃ + NO ₂	nM	140 \pm 13
PO ₄	nM	8 \pm 1
DOC	nM	74 \pm 1
Fe	nM	8.5 \pm 1.8
Zn	nM	8.7 \pm 2.1
Cu	nM	1.4 \pm 0.9
Bacterial abundance	Cells $\times 10^4$ /ml	350 \pm 15
Bacterial production	$\mu\text{g C} \cdot \text{L}^{-1} \cdot \text{hr}^{-1}$	1.41 \pm 0.08
N ₂ fixation	$\text{nmol N} \cdot \text{L}^{-1} \cdot \text{day}^{-1}$	0.11 \pm 0.02

added dust triggered a rapid (incubation with ¹⁵N₂ started 6 hr after enrichment) increase in N₂ fixation ($0.35 \pm 0.06 \text{ nmol N} \cdot \text{L}^{-1} \cdot \text{day}^{-1}$ and $0.29 \pm 0.06 \text{ nmol N} \cdot \text{L}^{-1} \cdot \text{day}^{-1}$, for February 2017 and May 2017, live-dust additions, respectively). These rates represent an approximately threefold increase in N₂ fixation rates compared to the untreated controls (one-way ANOVA, $P < 0.01$; Figure 2a). The slightly lower N₂ fixation rates measured in the May 2017 compared to the February 2017 mesocosm (one-way ANOVA, $P > 0.05$) may be attributed to the higher soluble NO₃ + NO₂ release from the May 2017 dust (by a factor of 3 compared to February 2017; Table 1) that may have inhibited the nitrogenase complex (Knapp, 2012). The differences in the measured N₂ fixation rates between the two live-dust additions compared to the no-addition control were maintained throughout the whole experiment with rates in February 2017 > May 2017 > "Control" (Figure 2a).

The differences in N₂ fixation following the aerosol addition can be explained by the contribution of nutrients from the aerosols, particularly P and/or DOC since Fe in the ambient NRS water is high to begin with (Table 2; Chase et al., 2006, 2011). A similar response has been reported previously for the North Atlantic where Fe and P addition from reagents or Saharan dust increased N₂ fixation rates (Mills et al., 2004), suggesting nutrient limitation relieve. In contrary, in the P-limited eastern Mediterranean Sea, Saharan dust addition led to a larger increase in N₂ fixation compared to similar artificial P addition (Ridame et al., 2011). The latter study hypothesized that these differences were attributed to leached trace-metals other than Fe because Fe concentrations are high in the Mediterranean surface water (as in the NRS). Our results, however, provide an alternative (not mutually exclusive) explanation, namely, that the differences between treatments may be explained by contribution of viable airborne diazotrophs that were leached off the dust particles.

The increase in N₂ fixation rates in all treatments was low, yet the differences between treatments were quantitatively measurable nonetheless (Figure 2a). Such low fixation rates are likely due to low abundances of diazotrophs in the water used for incubation (Foster et al., 2009; Post et al., 2002) or due to the competition with nondiazotrophic phytoplankton and microbes for the added nutrients and metals.

The differences in N₂ fixation rates between the live-dust and "killed-dust" aerosol amendments represent the contribution of airborne diazotrophs (Rahav, Ovadia, et al., 2016; Rahav, Paytan, et al., 2016). Our results demonstrate that airborne diazotrophy accounted for $\sim 0.10 \text{ nmol N} \cdot \text{L}^{-1} \cdot \text{day}^{-1}$ of the N₂ fixation rates measured after the addition of the February 2017 and May 2017 aerosols (Figure 2b). Similarly, the dust-leached macronutrients/micronutrients (i.e., P/DOC/trace metals and "UV-killed" dust) accounted for additional $0.10\text{--}0.20 \text{ nmol N} \cdot \text{L}^{-1} \cdot \text{day}^{-1}$ of the observed increase in N₂ fixation (Figure 2b). These N₂ fixation levels were overall similar to the in situ rates measured in our control mesocosms ($\sim 0.10 \text{ nmol N} \cdot \text{L}^{-1} \cdot \text{day}^{-1}$; Figure 2a) as well as to previously reported rates from the NRS (Foster et al., 2009; Rahav et al., 2015). Our measured airborne N₂ fixation rates were approximately twofold higher than those attributed to airborne diazotrophs in sterile seawater from the Eastern Mediterranean Sea reported by Rahav, Ovadia, et al. (2016), suggesting that the contribution of airborne diazotrophs may vary temporally depending on the aerosol source and the conditions at the deposition site. Airborne bacterial abundance, production, and β -Glu activity (calculated as the difference between the live-dust and killed-dust treatments) were all positively correlated with N₂ fixation (Figures 3a–3c). This suggests that airborne diazotrophs used the β -Glu pathway to utilize DOC (leached from the aerosols and/or from the ambient water) as an energy source for N₂ fixation. A recent study at the eastern Mediterranean Sea showed that β -Glu is used by diazotrophs to fulfill the high-energy needs required for sustaining N₂ fixation (Rahav, Giannetto, et al., 2016). Yet the bioavailability of DOC and the different

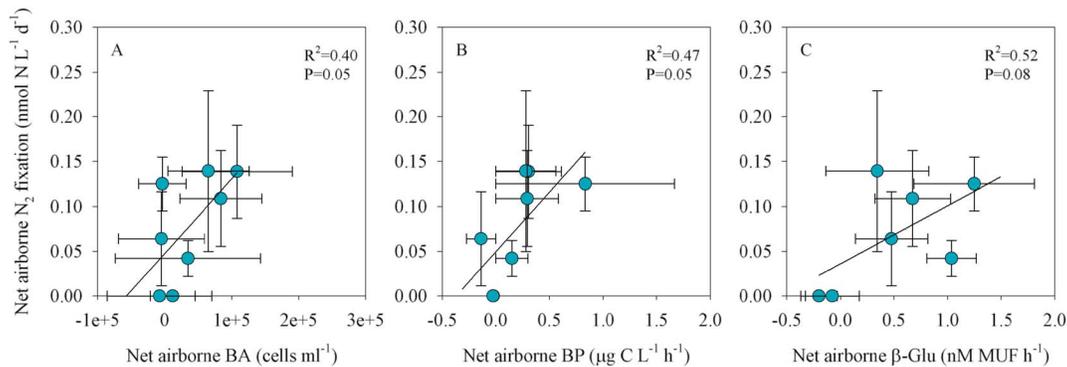


Figure 3. The relationship between (a) airborne N₂ fixation and airborne bacterial abundance (BA), (b) airborne bacterial production (BP), and (c) airborne β-glucosidase activity (β-Glu) during the mesocosm experiment ($n = 8$). The net airborne contribution was calculated by subtracting the values of the “killed-dust” from those of the “live-dust” treatments (of February and May 2017).

cellular pathways used by different heterotrophs to utilize DOC (i.e., α-glucosidase and aminopeptidase) should be further examined specifically for heterotrophic diazotrophs in seawater/aerosols.

In accordance with the measured airborne N₂ fixation rates (Figure 2b), two *nifH* sequences were amplified from the dust samples, which were related to cluster III proteobacterial diazotrophs and included nitrogenases from sulphate reducers (Deltaproteobacteria) and purple sulfur bacteria. Such sequences were previously found in dust samples collected at the NRS (Foster et al., 2009) as well as in seawater *nifH* sequences from the geographically related eastern Mediterranean Sea (Man-Aharonovich et al., 2007; Yogev et al., 2011). Foster et al. (2009) also reported on cluster II *nifH* gene sequences in their collected dust samples, which we did not observe here. Currently, we cannot say whether the low diazotrophic diversity in our samples is related to the aerosol sources and their route prior to deposition (Figure 1) or to other factors selecting specific airborne diazotrophs. Nevertheless, these results support our observation that airborne diazotrophs contributed to N₂ fixation following dust deposition in the NRS.

Assuming that airborne diazotrophs are responsible for ~30% of the N₂ fixation following dust events as shown here (~0.10 nmol N · L⁻¹ · d⁻¹; Figure 2b), ~15 dust storm events per year (Torfstein et al., 2017), and a receiving volume of 3.31×10^9 m³ (upper 10 m of the water column corresponding to the mixed layer depth and the surface area of the Gulf of Aqaba), airborne N₂ fixation can potentially contribute ~0.7-ton N to the Gulf per year. These values are similar to the annual N input from urban discharge from storm water (~0.6-ton N) and are ~5% of groundwater discharge (~13.5-ton N) in the Gulf of Aqaba basin (Thetis, SpA, 2013). Further, it is to be noted that the N input by airborne diazotrophy during five-day storm as tested here (≈0.65 nmol N mg/aerosol) is significantly lower than the N released from the same aerosol particles (20–60 nmol mg/aerosol; Table 1). Thus, chemically leached N may “support” more new production than airborne diazotrophy during dust events. Consistent with these estimates, the calculated new production due to airborne N₂ fixation is ~1–2% of the total primary productivity in the NRS (Iluz et al., 2009; Rahav et al., 2015). This low contribution is in agreement with other studies conducted in oligotrophic environments/provinces where N₂ fixation contributed <5% to primary production (Berman-Frank & Rahav, 2012; Bonnet et al., 2011; Rahav, Herut, Levi, et al., 2013; Rahav, Herut, Stambler, et al., 2013). Nevertheless, airborne diazotrophs are important contributors to total N₂ fixation in the NRS and we expect that this is also the case in other marine environments (especially oligotrophic) affected by high aerosol deposition (North Atlantic Ocean, Arabian Sea, and the Mediterranean Sea; Sohm et al., 2011), thereby playing a role in global N₂ fixation. Future studies aimed at understanding the spatiotemporal activity of airborne diazotrophs, including their diversity and activity, are needed.

4. Conclusions

Our results demonstrate that the deposition of aerosols rich in mineral dust may supplement the surface ocean with bioavailable N via three, not mutually exclusive, processes: (1) release of N from aerosol particles upon interaction with the water (such as NO₃; Guieu et al., 2014; Herut et al., 2002), (2) indirectly by providing limiting nutrients to promote in situ diazotrophy (i.e., Fe and P; Mills et al., 2004; Rahav et al., 2015; Rahav,

Shun-Yan, et al., 2016), and (3) the delivery of active airborne diazotrophs to receiving waters. These airborne diazotrophs may contribute ~50% of the net increase in N₂ fixation following aerosol deposition (Figure 2), thereby playing an ecologically important role in the oligotrophic NRS and possibly in other marine provinces subjected to high atmospheric deposition. It is suggested that due to climate change, increasing temperatures together with decreasing relative humidity will lead to increased dust emissions in the Middle East area (Klingmüller et al., 2016; Lelieveld et al., 2012) and consequently supply more nutrients and possibly airborne diazotrophs (and other bacteria/archaea) into surface waters impacted by high dust deposition.

Acknowledgments

This study was supported by the Israel Science Foundation (grant 1211/17) to B. H and E. R and by the NSF-OCE (grant 0850467) to A. P. Y. G. was supported by Banca del Monte di Lombardia Foundation. We would like to thank Wan-Chen Tu for her contribution on the trace metal analysis. The authors thank the personnel in the Inter-University Institute for Marine Sciences in Eilat (IUI).

References

- Banks, J. R., Brindley, H. E., Stenchikov, G., & Schepanski, K. (2017). Satellite retrievals of dust aerosol over the Red Sea and the Persian Gulf (2005–2015). *Atmospheric Chemistry and Physics*, 17(6), 3987–4003. <https://doi.org/10.5194/acp-17-3987-2017>
- Benavides, M., Martias, C., Elifantz, H., Berman-Frank, I., Dupouy, C., & Bonnet, S. (2018). Dissolved organic matter influences N₂ fixation in the New Caledonian lagoon (Western Tropical South Pacific). *Frontiers in Marine Science*, 5, 1–11. <https://doi.org/10.3389/fmars.2018.00089>
- Berman-Frank, I., Lundgren, P., Chen, Y.-B., Küpper, H., Kolber, Z., Bergman, B., & Falkowski, P. (2001). Segregation of nitrogen fixation and oxygenic photosynthesis in the marine cyanobacterium *Trichodesmium*. *Science*, 294(5546), 1534–1537. <https://doi.org/10.1126/science.1064082>
- Berman-Frank, I., Quigg, A., Finkel, Z. V., Irwin, A. J., & Haramaty, L. (2007). Nitrogen-fixation strategies and Fe requirements in cyanobacteria. *Limnology and Oceanography*, 52(5), 2260–2269. <https://doi.org/10.4319/lo.2007.52.5.2260>
- Berman-Frank, I., & Rahav, E. (2012). Nitrogen fixation as a source for new production in the Mediterranean Sea: A review. In *Life in the Mediterranean Sea: A Look at Habitat Changes* (1st ed., pp. 199–226). New York: Nova Science Publishers.
- Bonnet, S., Grosso, O., & Moutin, T. (2011). Planktonic dinitrogen fixation along a longitudinal gradient across the Mediterranean Sea during the stratified period (BOUM cruise). *Biogeosciences*, 8(8), 2257–2267. <https://doi.org/10.5194/bg-8-2257-2011>
- Capone, D. G., Burns, J. A., Montoya, J. P., Subramaniam, A., Mahaffey, C., Gunderson, T., et al. (2005). Nitrogen fixation by *Trichodesmium* spp.: An important source of new nitrogen to the tropical and subtropical North Atlantic Ocean. *Global Biogeochemical Cycles*, 19, GB2024. <https://doi.org/10.1029/2004GB002331>
- Chase, Z., Paytan, A., Beck, A., Biller, D., Bruland, K., Measures, C., & Sañudo-Wilhelmy, S. (2011). Evaluating the impact of atmospheric deposition on dissolved trace-metals in the Gulf of Aqaba, Red Sea. *Marine Chemistry*, 126(1–4), 256–268. <https://doi.org/10.1016/j.marchem.2011.06.005>
- Chase, Z., Paytan, A., Johnson, K. S., Street, J., & Chen, Y. (2006). Input and cycling of iron in the Gulf of Aqaba, Red Sea. *Global Biogeochemical Cycles*, 20, GB3017. <https://doi.org/10.1029/2005GB002646>
- Chen, Y., Paytan, A., Chase, Z., Measures, C., Beck, A. J., Sañudo-Wilhelmy, S. A., & Post, A. F. (2008). Sources and fluxes of atmospheric trace elements to the Gulf of Aqaba, Red Sea. *Journal of Geophysical Research*, 113, 1–13. <https://doi.org/10.1029/2007JD009110>
- Dishon, G., Dubinsky, Z., Caras, T., Rahav, E., Bar-Zeev, E., Tzuber, Y., & Iluz, D. (2012). Optical habitats of ultraplankton groups in the Gulf of Eilat (Aqaba), Northern Red Sea. *International Journal of Remote Sensing*, 33(9), 2683–2705. <https://doi.org/10.1080/01431161.2011.619209>
- Dyhrman, S. T., & Haley, S. T. (2006). Phosphorus scavenging in the unicellular marine diazotroph *Crocosphaera watsonii*. *Applied and Environmental Microbiology*, 72(2), 1452–1458. <https://doi.org/10.1128/AEM.72.2.1452-1458.2006>
- Foster, R. A., Paytan, A., & Zehr, J. P. (2009). Seasonality of N₂ fixation and *nifH* gene diversity in the Gulf of Aqaba (Red Sea). *Limnology and Oceanography*, 54(1), 219–233. <https://doi.org/10.4319/lo.2009.54.1.0219>
- Gruber, N., & Galloway, J. N. (2008). An Earth-system perspective of the global nitrogen cycle. *Nature*, 451(7176), 293–296. <https://doi.org/10.1038/nature06592>
- Gruber, N., & Sarmiento, J. L. (1997). Global patterns of marine nitrogen fixation and denitrification. *Global Biogeochemical Cycles*, 11, 235–266.
- Guiu, C., Aumont, O., Paytan, A., Bopp, L., Law, C. S., Mahowald, N., et al. (2014). The significance of the episodic nature of atmospheric deposition to low nutrient low chlorophyll regions. *Global Biogeochemical Cycles*, 28, 1179–1198. <https://doi.org/10.1002/2014GB004852>
- Herut, B., Kress, N., & Tibor, G. (2002). The use of hyper-spectral remote sensing in compliance monitoring of water quality (phytoplankton and suspended particles) at “hot spot” areas (Mediterranean coast of Israel). *Fresenius Environmental Bulletin*, 11(10A), 782–787.
- Herut, B., Krom, M. D. D., Pan, G., & Mortimer, R. (1999). Atmospheric input of nitrogen and phosphorus to the Southeast Mediterranean: Sources, fluxes, and possible impact. *Limnology and Oceanography*, 44(7), 1683–1692. <https://doi.org/10.4319/lo.1999.44.7.1683>
- Herut, B., Rahav, E., Tsagaraki, T. M., Giannakourou, A., Tsiola, A., Psarra, S., et al. (2016). The potential impact of Saharan dust and polluted aerosols on microbial populations in the East Mediterranean Sea, an overview of a mesocosm experimental approach. *Frontiers in Marine Science*, 3, 1–16. <https://doi.org/10.3389/fmars.2016.00226>
- Ho, T. Y., Chien, C. T., Wang, B. N., & Siriraks, A. (2010). Determination of trace metals in seawater by an automated flow injection ion chromatograph pretreatment system with ICPMS. *Talanta*, 82(4), 1478–1484. <https://doi.org/10.1016/j.talanta.2010.07.022>
- Hoppe, H. G. (1983). Significance of exoenzymatic activities in the ecology of brackish water: Measurements by means of methylumbelliferyl-substrates. *Marine Ecology Progress Series*, 11, 299–308. <https://doi.org/10.3354/meps011299>
- Iluz, D., Dishon, G., Capuzzo, E., Meeder, E., Astoreca, R., Montecino, V., et al. (2009). Short-term variability in primary productivity during a wind-driven diatom bloom in the Gulf of Eilat (Aqaba). *Aquatic Microbial Ecology*, 56(2–3), 205–215. <https://doi.org/10.3354/ame01321>
- Jish Prakash, P., Stenchikov, G., Kalenderski, S., Osipov, S., & Bangalath, H. (2015). The impact of dust storms on the Arabian Peninsula and the Red Sea. *Atmospheric Chemistry and Physics*, 15(1), 199–222. <https://doi.org/10.5194/acp-15-199-2015>
- Klingmüller, K., Pozzer, A., Metzger, S., Stenchikov, G. L., & Lelieveld, J. (2016). Aerosol optical depth trend over the Middle East. *Atmospheric Chemistry and Physics*, 16(8), 5063–5073. <https://doi.org/10.5194/acp-16-5063-2016>
- Knapp, A. N. (2012). The sensitivity of marine N₂ fixation to dissolved inorganic nitrogen. *Frontiers in Microbiology*, 3, 374. <https://doi.org/10.3389/fmicb.2012.00374>
- Lelieveld, J., Hadjinicolaou, P., Kostopoulou, E., Chenoweth, J., El Maayar, M., Giannakopoulos, C., et al. (2012). Climate change and impacts in the eastern Mediterranean and the Middle East. *Climatic Change*, 114(3–4), 667–687. <https://doi.org/10.1007/s10584-012-0418-4>
- Man-Aharonovich, D., Kress, N., Bar-Zeev, E., Berman-Frank, I., & Bèjà, O. (2007). Molecular ecology of *nifH* genes and transcripts in the eastern Mediterranean Sea. *Environmental Microbiology*, 9(9), 2354–2363. <https://doi.org/10.1111/j.1462-2920.2007.01353.x>
- Marañón, E., Fernández, A., Mouriño-Carballido, B., Martínez-García, S., Teira, E., Cermeño, P., et al. (2010). Degree of oligotrophy controls the response of microbial plankton to Saharan dust. *Limnology and Oceanography*, 55(6), 2339–2352. <https://doi.org/10.4319/lo.2010.55.6.2339>

- Meeder, E., Mackey, K., Paytan, A., Shaked, Y., Iluz, D., Stambler, N., et al. (2012). Nitrite dynamics in the open ocean—Clues from seasonal and diurnal variations. *Marine Ecology Progress Series*, 453(2), 11–26. <https://doi.org/10.3354/meps09525>
- Mills, M. M., Ridame, C., Davey, M., La Roche, J., & Geider, R. J. (2004). Iron and phosphorus co-limit nitrogen fixation in the eastern tropical North Atlantic. *Nature*, 429(6989), 292–294. <https://doi.org/10.1038/nature02550>
- Mohr, W., Großkopf, T., Wallace, D. W. R., & Laroche, J. (2010). Methodological underestimation of oceanic nitrogen fixation rates. *PLoS One*, 5(9), 1–7. <https://doi.org/10.1371/journal.pone.0012583>
- Moore, C. M., Mills, M. M., Achterberg, E. P., Geider, R. J., LaRoche, J., Lucas, M. I., et al. (2009). Large-scale distribution of Atlantic nitrogen fixation controlled by iron availability. *Nature Geoscience*, 2(12), 867–871. <https://doi.org/10.1038/ngeo667>
- Post, A. F., Dedej, Z., Gottlieb, R., Li, H., Thomas, D. N., El-Absawi, M., et al. (2002). Spatial and temporal distribution of *Trichodesmium* spp. in the stratified Gulf of Aqaba, Red Sea. *Marine Ecology Progress Series*, 239, 241–250. <https://doi.org/10.3354/meps239241>
- Rahav, E., & Bar-Zeev, E. (2017). Sewage outburst triggers *Trichodesmium* bloom and enhance N₂ fixation rates. *Scientific Reports*, 7(1), 4367. <https://doi.org/10.1038/s41598-017-04622-8>
- Rahav, E., Giannetto, M., & Bar-Zeev, E. (2016). Contribution of mono and polysaccharides to heterotrophic N₂ fixation at the eastern Mediterranean coastline. *Scientific Reports*, 6(1), 27858. <https://doi.org/10.1038/srep27858>
- Rahav, E., Herut, B., Levi, A., Mulholland, M. R., & Berman-Frank, I. (2013). Springtime contribution of dinitrogen fixation to primary production across the Mediterranean Sea. *Ocean Science*, 9(3), 489–498. <https://doi.org/10.5194/os-9-489-2013>
- Rahav, E., Herut, B., Mulholland, M., Belkin, N., Elifantz, H., & Berman-Frank, I. (2015). Heterotrophic and autotrophic contribution to dinitrogen fixation in the Gulf of Aqaba. *Marine Ecology Progress Series*, 522, 67–77. <https://doi.org/10.3354/meps11143>
- Rahav, E., Herut, B., Stambler, N., Bar-Zeev, E., Mulholland, M. R., & Berman-Frank, I. (2013). Uncoupling between dinitrogen fixation and primary productivity in the eastern Mediterranean Sea. *Journal of Geophysical Research: Biogeosciences*, 118, 195–202. <https://doi.org/10.1002/jgrg.20023>
- Rahav, E., Ovadia, G., Paytan, A., & Herut, B. (2016). Contribution of airborne microbes to bacterial production and N₂ fixation in seawater upon aerosol deposition. *Geophysical Research Letters*, 43, 719–727. <https://doi.org/10.1002/2015GL066898>
- Rahav, E., Paytan, A., Chien, C. T., Ovadia, G., Katz, T., & Herut, B. (2016). The impact of atmospheric dry deposition associated microbes on the southeastern mediterranean sea surface water following an intense dust storm. *Frontiers in Marine Science*, 3, 127. <https://doi.org/10.3389/fmars.2016.00127>
- Rahav, E., Shun-Yan, C., Cui, G., Liu, H., Tsagaraki, T. M., Giannakourou, A., et al. (2016). Evaluating the impact of atmospheric depositions on springtime dinitrogen fixation in the Cretan Sea (eastern Mediterranean)—A mesocosm approach. *Frontiers in Marine Science*, 3, 1–13. <https://doi.org/10.3389/fmars.2016.00180>
- Ridame, C., Guieu, C., & L'Helguen, S. (2013). Strong stimulation of N₂ fixation in oligotrophic Mediterranean Sea: Results from dust addition in large *in situ* mesocosms. *Biogeosciences*, 10(11), 7333–7346. <https://doi.org/10.5194/bg-10-7333-2013>
- Ridame, C., Le Moal, M., Guieu, C., Ternon, E., Biegala, I. C., L'Helguen, S., & Pujo-Pay, M. (2011). Nutrient control of N₂ fixation in the oligotrophic Mediterranean Sea and the impact of Saharan dust events. *Biogeosciences*, 8(9), 2773–2783. <https://doi.org/10.5194/bg-8-2773-2011>
- Santinelli, C., Hansell, D. A., & Ribera d'Alcalà, M. (2013). Influence of stratification on marine dissolved organic carbon (DOC) dynamics: The Mediterranean Sea case. *Progress in Oceanography*, 119, 68–77. <https://doi.org/10.1016/j.pocean.2013.06.001>
- Sanudo-Wilhelmy, S., Kustka, A. B., Gobler, C. J., Hutchins, D. A., Yang, M., Lwiza, K., et al. (2001). Phosphorus limitation of nitrogen fixation by *Trichodesmium* in the central Atlantic Ocean. *Nature*, 411(6833), 66–69. <https://doi.org/10.1038/35075041>
- Simon, M., Alldredge, A., & Azam, F. (1990). Bacterial carbon dynamics on marine snow. *Marine Ecology Progress Series*, 65(3), 205–211. <https://doi.org/10.3354/meps065205>
- Sohm, J., Webb, A., & Capone, D. G. (2011). Emerging patterns of marine nitrogen fixation. *Nature Reviews. Microbiology*, 9(7), 499–508. <https://doi.org/10.1038/nrmicro2594>
- Thetis, SpA (2013). Red-Sea study, Final report. Red Sea-Dead Sea water conveyance study program reports.
- Torfstein, A., Teutsch, N., Tirosh, O., Shaked, Y., Rivlin, T., Zipori, A., et al. (2017). Chemical characterization of atmospheric dust from a weekly time series in the north Red Sea between 2006 and 2010. *Geochimica et Cosmochimica Acta*, 211, 373–393. <https://doi.org/10.1016/j.gca.2017.06.007>
- Wang, B.-S., Lee, C.-P., & Ho, T.-Y. (2014). Trace metal determination in natural waters by automated solid phase extraction system and ICP-MS: The influence of low level Mg and Ca. *Talanta*, 128, 337–344. <https://doi.org/10.1016/j.talanta.2014.04.077>
- Yogev, T., Rahav, E., Bar-Zeev, E., Man-Aharonovich, D., Stambler, N., Kress, N., et al. (2011). Is dinitrogen fixation significant in the Levantine Basin, East Mediterranean Sea? *Environmental Microbiology*, 13(4), 854–871. <https://doi.org/10.1111/j.1462-2920.2010.02402.x>
- Zehr, J. P., & McReynolds, L. A. (1989). Use of degenerate oligonucleotides for amplification of the nifH gene from the marine cyanobacterium *Trichodesmium thiebautii*. *Applied and Environmental Microbiology*, 55(10), 2522–2526.