# Airborne Microbes Contribute to $N_2$ Fixation in Surface Water of the Northern Red Sea

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**Abstract** Desert dust storms are frequent in the Northern Red Sea region, providing nutrients (i.e., $PO_4$) and trace-metals (i.e., Fe) that may stimulate dinitrogen ($N_2$) 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 $N_2$ 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 $N_2$ 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 $N_2$ fixation ($0.35 \pm 0.06$ nmol $N \cdot L^{-1} \cdot day^{-1}$ and $0.29 \pm 0.06$ nmol $N \cdot L^{-1} \cdot day^{-1}$, 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 $N_2$ fixation measurement results. We postulate that the deposition of viable airborne diazotrophs may enhance $N_2$ fixation, especially in marine provinces subjected to high aerosol loads. We speculate that the relative contribution of airborne $N_2$ 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 $N_2$ 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 $N_2$ fixation using "live-dust" (release nutrients/trace metals and viable airborne microorganisms) and "UV-killed dust" (release only chemicals). Our results demonstrate that airborne $N_2$ 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 $N_2$ 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 ($N_2$) 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 $N_2$ 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 $N_2$ 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 $N_2$ 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 homogenously 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 µmol quanta $\cdot$ m$^{-2} \cdot$ 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.
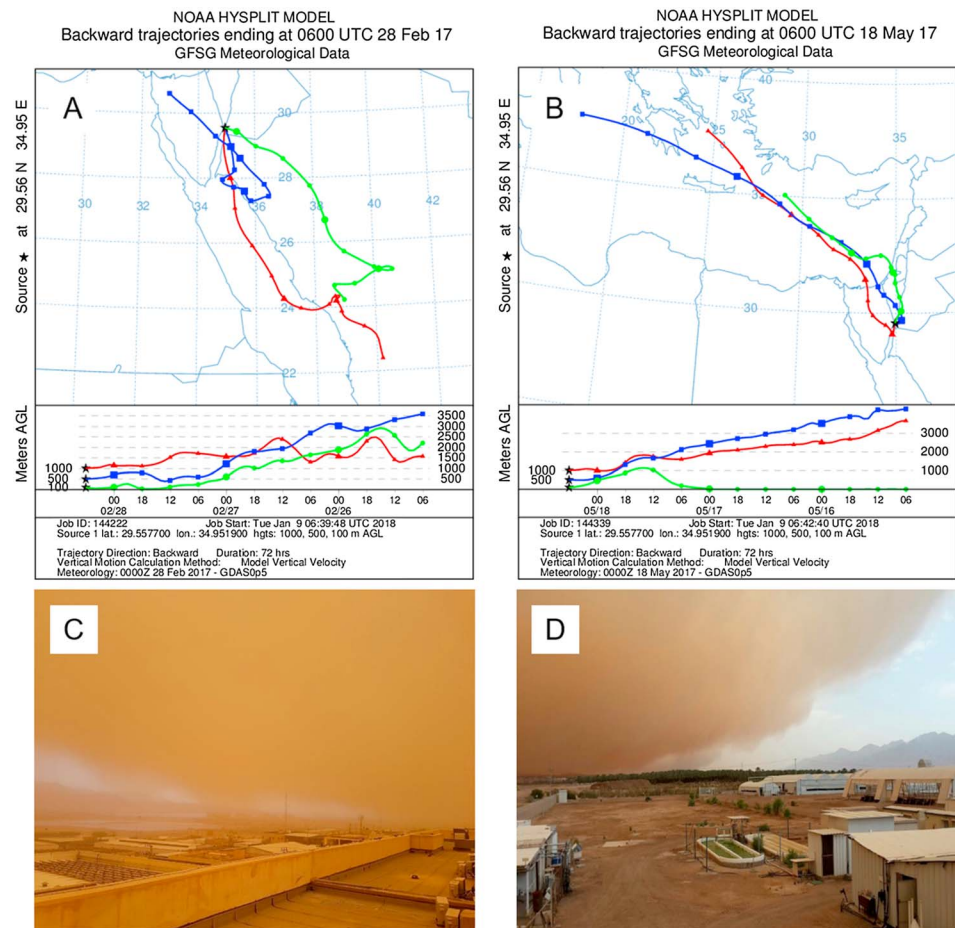
**Figure 1.** The origin of the aerosols used in the mesocosm experiment based on three-day back trajectories (100, 500, and 1,000 m altitude). Aerosols were collected at the study site (Northern Red Sea) in (a and c) February 2017 and (b and d) May 2017 during dust storms.

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 add 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 NO$_3$ + NO$_2$ and PO$_4$ by a photometric segmented flow method (Seal Analytical AA-3 system, Herut et al., 1999). The precision for NO$_3$ + 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 °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 ($N_2$) Fixation

$^{15}N_2$-enriched seawater (Mohr et al., 2010) was added to 4.6 L Nalgene bottles containing the different meso-cosm treatments (5% of the total volume). A recent study from the NRS showed that such addition yields the highest $N_2$ fixation rates without overdiluting the sample with $^{15}N_2$-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 over-night and analyzed using a CE Instruments NC2500 elemental analyzer interfaced to a Thermo-Finningan 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 hetero-trophic 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 $N_2$ 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 ± standard-deviation from three independent replicate mesocosms. The relationship between the $N_2$ 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 nutri-ent ($NO_3 + NO_2$, $PO_4$, and DOC) and trace element characteristics exhibited distinct chemical differences (Table 1). In the February 2017 sample, each milligram of aerosol contributed ~20 nmol $NO_3 + NO_2$ and ~2 nmol $PO_4$ 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 $NO_3 + NO_2$ (+34%) and ~2.4 nM $PO_4$ (+30%) for the May 2017 mesocosm, and ~16 nM $NO_3 + NO_2$ (+11%) and 1.6 nM $PO_4$ (+20%) for the February 2017 mesocosm (Tables 1 and 2). Addition of $PO_4$ was previously shown to stimulate $N_2$ fixation in the NRS (Foster et al., 2009; Rahav et al., 2015), while the low $NO_3 + NO_2$ 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
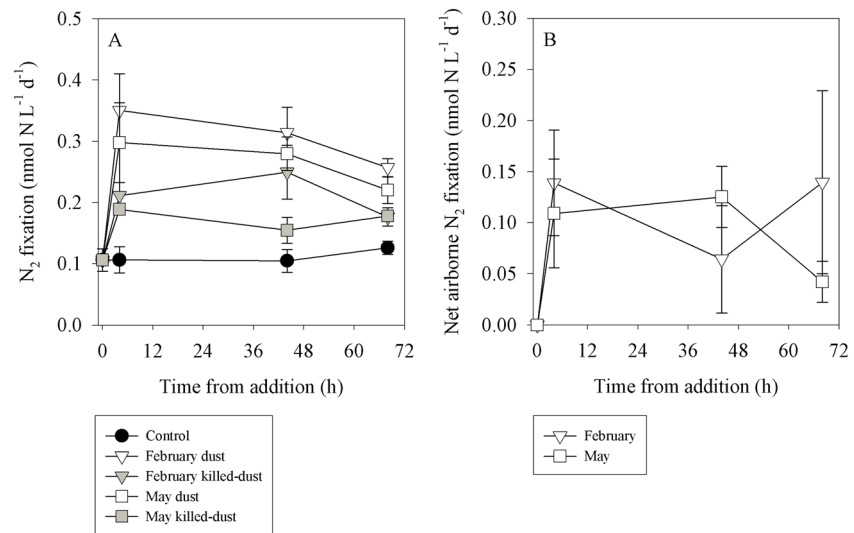
**Figure 2.** Temporal changes in volumetric (a) $N_2$ fixation rates and the (b) net airborne diazotrophic activity following dust additions (~0.8 mg/L) to surface seawater of the Northern Red Sea during July 2017. The following treatments were performed: dust collected in February 2017 (white triangle), 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 constitutes (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 ($0.11 \pm 0.02$ 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 ± 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 ± SD ($n = 3$) |
|---|---|---|
| $NO_3 + NO_2$ | nM | 140 ± 13 |
| $PO_4$ | nM | 8 ± 1 |
| DOC | nM | 74 ± 1 |
| Fe | nM | 8.5 ± 1.8 |
| Zn | nM | 8.7 ± 2.1 |
| Cu | nM | 1.4 ± 0.9 |
| Bacterial abundance | Cells $\times 10^4$/ml | 350 ± 15 |
| Bacterial production | $\mu g\ C \cdot L^{-1} \cdot hr^{-1}$ | 1.41 ± 0.08 |
| $N_2$ fixation | $nmol\ N \cdot L^{-1} \cdot day^{-1}$ | 0.11 ± 0.02 |

added dust triggered a rapid (incubation with $^{15}N_2$ started 6 hr after enrichment) increase in $N_2$ fixation ($0.35 \pm 0.06$ nmol N $\cdot$ L$^{-1} \cdot$ day$^{-1}$ and $0.29 \pm 0.06$ nmol N $\cdot$ L$^{-1} \cdot$ day$^{-1}$, for February 2017 and May 2017, live-dust additions, respectively). These rates represent an approximately threefold increase in $N_2$ fixation rates compared to the untreated controls (one-way ANOVA, $P < 0.01$; Figure 2a). The slightly lower $N_2$ 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 $NO_3 + NO_2$ 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 $N_2$ fixation rates between the two live-dust additions compared to the no-addition control were maintained throughout the whole experiment with rates in February2017 > May 2017 > "Control" (Figure 2a).

The differences in $N_2$ 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 $N_2$ 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 $N_2$ 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 $N_2$ 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 $N_2$ 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 ~0.10 nmol N $\cdot$ L$^{-1} \cdot$ day$^{-1}$ of the $N_2$ 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 0.10–0.20 nmol N $\cdot$ L$^{-1} \cdot$ day$^{-1}$ of the observed increase in $N_2$ fixation (Figure 2b). These $N_2$ fixation levels were overall similar to the in situ rates measured in our control mesocosms (~0.10 nmol N $\cdot$ L$^{-1} \cdot$ 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 $N_2$ 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 $N_2$ 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 $N_2$ 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 $N_2$ fixation (Rahav, Giannetto, et al., 2016). Yet the bioavailability of DOC and the different
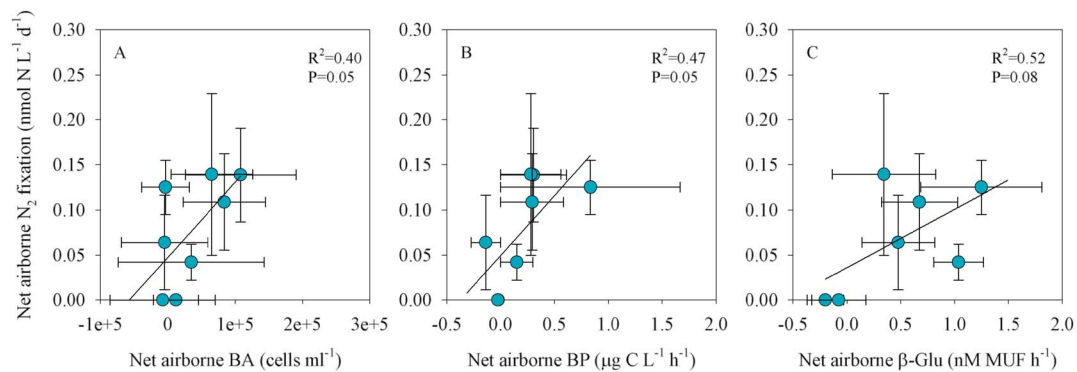
**Figure 3.** The relationship between (a) airborne $N_2$ fixation and airborne bacterial abundance (BA), (b) airborne bacterial production (BP), and (c) airborne β-glucosidase activity (β-Glu) during the mesocosm experiment ($n = 8$). The net airborne contribution was calculated by subtracting the values 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 $N_2$ 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 observed 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 $N_2$ fixation following dust deposition in the NRS.

Assuming that airborne diazotrophs are responsible for ~30% of the $N_2$ 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 $3.31 \times 10^9$ m$^3$ (upper 10 m of the water column corresponding to the mixed layer depth and the surface area of the Gulf of Aqaba), airborne $N_2$ 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 $N_2$ 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 $N_2$ 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 $N_2$ 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 $N_2$ 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 $NO_3$; 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,

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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 $N_2$ 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.

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