Contribution of airborne microbes to bacterial production and N_2 fixation in seawater upon aerosol deposition

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Abstract Aerosol deposition may supply a high diversity of airborne microbes, which can affect surface microbial composition and biological production. This study reports a diverse microbial community associated with dust and other aerosol particles, which differed significantly according to their geographical air mass origin. Microcosm bioassay experiments, in which aerosols were added to sterile (0.2 μm filtered and autoclaved) SE Mediterranean Sea (SEMS) water, were performed to assess the potential impact of airborne bacteria on bacterial abundance, production, and N_2 fixation. Significant increase was observed in all parameters within a few hours, and calculations suggest that airborne microbes can account for one third in bacterial abundance and 50–100% in bacterial production and N_2-fixation rates following dust/aerosol amendments in the surface SEMS. We show that dust/aerosol deposition can be a potential source of a wide array of microorganisms, which may impact microbial composition and food web dynamics in oligotrophic marine systems such as the SEMS.

1. Introduction

Low-nutrient low-chlorophyll (LNLC) marine provinces occupy 50–60% of the ocean's surface areas [*Cullen and Boyd*, 2008; *Steinacher et al.*, 2010]. These oligotrophic regions are usually dominated by small-size pico-phytoplankton [*Uitz et al.*, 2010] and by fast microbial recycling of nutrients [*Azam and Malfatti*, 2007; *Buchan et al.*, 2014]. Future climate change scenarios predict warmer, more stratified, and larger LNLC regions, reducing nutrient availability to the photic zone [*Polovina et al.*, 2008; *Steinacher et al.*, 2010; *Gruber et al.*, 2011].

The supply of new nutrients from external sources such as atmospheric deposition to LNLC regions has been studied through model simulations [*Mahowald*, 2007; *Guieu et al.*, 2014], as well as laboratory and onboard experiments simulating dust/aerosol addition scenarios [*Mills et al.*, 2004; *Mackey et al.*, 2012; *Guieu et al.*, 2014]. These studies show that atmospheric inputs may provide limiting macronutrients (nitrate and phosphate) and micronutrients (trace metals) required to sustain productivity (reviewed in *Guieu et al.* [2014]) or can supply toxic metals and other pollutants [*Paytan et al.*, 2009; *Jordi et al.*, 2012].

In addition to supplying chemical constituents, aerosols also supply a wide array of airborne microbes [*Burrows et al.*, 2009; *Reche et al.*, 2009; *Womack et al.*, 2010; *Polymenakou*, 2012; *Peter et al.*, 2014; *Katra et al.*, 2014]. A considerable fraction (1–25%) of these microbes remains viable during transport [*Womack et al.*, 2010; *Polymenakou*, 2012]. Despite this, only a handful of studies focus on ecological implications of airborne microbes in marine ecosystems [*Kellogg and Griffin*, 2006; *Smith*, 2013].

The SE Mediterranean Sea (SEMS) is a stratified ultraoligotrophic LNLC environment [*Krom et al.*, 1991; *Bosc et al.*, 2004; *Rahav et al.*, 2013a; *Raveh et al.*, 2015], with extremely low surface dissolved inorganic phosphorus and nitrogen concentrations [*Kress and Herut*, 2001; *Kress et al.*, 2014]. One of the main external sources for nutrients in this LNLC system is atmospheric depositions [*Herut et al.*, 1999; *Guerzoni et al.*, 1999; *Koçak et al.*, 2010; *Léon et al.*, 2015]. Previous studies showed that nutrient release from aerosol deposition triggered an increase in primary and bacterial production rates and caused a shift in the relative abundance of small-size cyanobacterial communities [*Herut et al.*, 2005; *Ternon et al.*, 2011; *Guieu et al.*, 2014].

To date, the role of airborne microbes play in the LNLC SEMS, which is subject to high atmospheric dust/aerosol deposition rates [*Dayan et al.*, 1991; *Ganor et al.*, 2010], is unclear. In this study, we characterized the genetic fingerprinting of microorganisms attached to aerosols from representative storm events of

different geographical origins (Sahara, Eastern Europe, Middle East), collected between 2006 and 2012. Aerosol (dust) addition bioassay experiments in sterile seawater were used to evaluate the viability of airborne bacteria and their heterotrophic production and dinitrogen (N_2) fixation rates.

2. Methods

2.1. Aerosol Collection

Aerosols from seven dust storm events were collected using a high-volume sampler (at a flow rate of $42 \, m^3 \, h^{-1}$ for 12–60 h [*Herut et al.*, 2002]) located on the roof of the Israel National Institute of Oceanography (latitude 32.28°N, longitude 34.95°E), between 2006 and 2012, representing different geographical origins. Aerosols were collected on preweighted, acid-washed Whatman 41-filters (high-quality cotton linters, GE Healthcare). An additional dust sample was collected during a dust storm event using precleaned glass plates at the same site on February 2015. Back trajectories arriving at the study site have historically been divided into up to six sectors [i.e., *Koçak et al.*, 2004, 2005]. However, for the current study, a simpler three sector categorization was adopted (Figure 1a): (i) northern (East Europe) airmasses (relatively lower crustal to anthropogenic contributions), (ii) southwestern high crustal (Sahara desert) air masses, and (iii) southern high crustal (Middle East) air masses. Three-day back trajectories arriving at 500, 1000, and 3000 m altitude levels were calculated for each sample, commencing at 10:00 UTC using the Hybrid Single-Particle Lagrangian Integrated Trajectory (HYSPLIT) model from the Air-Resources Laboratory.

2.2. Aerosol Bioassay Experiments

Nine aerosol-enrichment microcosms (in triplicates) were carried out in 1-L acid washed polycarbonate Nalgene bottles using sterile (0.2 μm filtered and autoclaved-killed) surface SEMS water. Subsamples ($2.5 \, cm^2$) from each of the seven aerosol filters were added to the microcosm bottles, which were shaken and put in an outdoor pool with seawater flow-through to maintain ambient temperature and covered with a neutral density screening to simulate ambient light for 48 h. These additions represent $1.5–1.8 \, mg \, L^{-1}$ of aerosol concentrations. Blank treatments were carried out, containing either an acid-washed Whatman-41 filter or UV-killed (24 h) aerosols. Subsamples of water (20 mL) from each incubation bottle were collected for bacterial production and abundance measurements 0.5, 2, 4, 8, 24, 30, and 48 h after aerosol addition.

Additional bioassay microcosm experiment was conducted (in triplicate) using dust collected by glass plate in February 2015, which was originated in the Sahara desert (dash line, Figure 1a). Dust was added ($1.5 \, mg \, L^{-1}$) to 4.5 L acid washed polycarbonate Nalgene into sterile surface SEMS water as described above. After 48 h incubations under ambient light and temperature conditions, the treatments were sampled for bacterial production, bacterial abundance, and dinitrogen (N_2) fixation.

2.3. DNA Extractions, High-Throughput Phylogenetic Analyses, and Sequence Analyses

Community genomic nucleic acids were extracted from the seven dust samples (Table S1 in the supporting information) using the phenol-chloroform method modified according to *Massana et al.* [1997]. The universal eubacterial primers 27 F and 338R were used to amplify the 16S rRNA gene region. Polymerase chain reaction products for the 16S fractions were sequenced to get a representative view of the bacterial community composition in each filter using Roche 454 FLX titanium instruments and reagents. Sequences were processed and analyzed using "quantitative insights into microbial ecology" (QIIME), open-source software pipeline [*Caporaso et al.*, 2010]. See supporting information for further details.

Bray-Curtis dissimilarity index for beta-diversity comparison between relative operational taxonomic unit (OTU) across aerosol samples was calculated with the QIIME "beta_diversity.py" script [*Langille et al.*, 2013].

2.4. Bacterial Abundance

Samples (1 mL) for bacterial abundance were collected, fixed with 50% glutaraldehyde (0.15% final conc. v/v., Sigma G7651), and incubated in room temperature for 10 min and quickly frozen in liquid nitrogen. Prior to counting, samples were fast thawed at 37°C and stained with 0.5 nM SYTO9 (Applied-Biosystems) in the dark for 15 min [*Vaulot and Marie*, 1999]. Samples were analyzed with an Attune acoustic focusing flow cytometer (Applied-Biosystems) equipped with a syringe-based fluidic system at a wavelength of 520 nm and a flow rate of $25 \, μL \, min^{-1}$. Size standard was set using 0.93 μm beads (Polyscience).
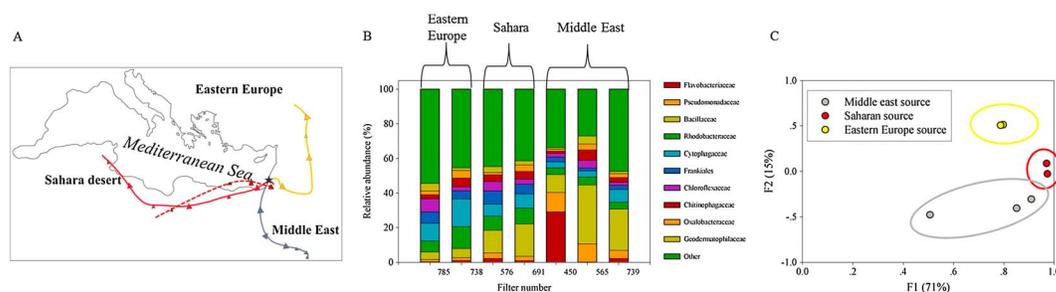
Figure 1. (a) Representative air mass back trajectories analyses derived from backward trajectories model NOAA/ARL HYSPLIT-4 (www.noaa.gov) showing the course and origin of the aerosols used in this study over the course of three days prior its collection at 500 m above sea level. Aerosols were collected between 2006–2012 (line colors) and on February 2015 (dotted line). The different colors represent the air mass trajectories, the Middle East (gray, filters #450, 565, and 739), Sahara desert (red, filters #576, 691, and dust collected in February 2015), and Eastern Europe (yellow, filters #785 and 738). (b) Taxonomic distributions of 16S rRNA gene analyses (family level) retrieved from the different aerosol samples, and (c) the genetic discrimination of bacterial phylotypes based on principle component analysis of the different dust sources. A Bray-Curtis dissimilarity index for beta-diversity comparison between relative OTUs across the different aerosol samples is shown in Table S2.

2.5. Bacterial Production

Rates were estimated using the ^{3}H-leucine (Amersham, specific activity: 160 Ci mmol^{-1}) incorporation method [*Simon et al.*, 1990]. A conversion factor of 1.5 kg C mol^{-1} leucine incorporated was used, assuming an isotopic dilution of 2.0 [*Simon et al.*, 1989]. See supporting information for further details.

2.6. Dinitrogen (N$_2$) Fixation

Rates of N$_2$ fixation were measured only for the experiment with dust collected in February 2015 (originated from the Sahara desert) using the newly developed ^{15}N$_2$-enriched seawater method [*Mohr et al.*, 2010] with minor modifications [*Rahav et al.*, 2013a]. See supporting information for further details.

2.7. Statistics

Principle component analysis of the rRNA diversity from the different aerosol sources were generated using the XLSTAT software. Changes in bacterial abundance, production, and N$_2$ fixation rates in the different aerosol treatments were evaluated using a one-way analysis of variance (ANOVA), followed by a Fisher LSD multiple comparison post hoc test with a confidence of 95% ($\alpha = 0.05$), using the XLSTAT software.

3. Results and Discussion

Dust and aerosol deposition worldwide range from 0.5 to 5.0 billion tons [*Perkins*, 2001], mostly from the Sahara and Sahel deserts (>50% [*Prospero and Lamb*, 2003]). The SEMS, being relatively close to desert sources, is frequently subjected to high inputs of dry atmospheric depositions [*Guerzoni et al.*, 1999; *Herut et al.*, 1999, 2002], ranging from 1 to 50 g m^{-2} yr^{-1} [*Lawrence and Neff*, 2009]. These atmospheric inputs will likely become even greater in the near future when considering desertification and human-induced activities [*Prospero and Lamb*, 2003; *Neff et al.*, 2008]. In this study, aerosol samples collected at the SEMS representing relatively large continental atmospheric deposition events were studied (Table S1). These samples were either impacted by anthropogenic sources (e.g., Eastern Europe samples) or represent largely particles from natural land source (i.e., Middle East and Sahara).

3.1. The Diversity and Functionality of Heterotrophic Airborne Microorganisms

Heterotrophic airborne microbial community, derived from 16S rRNA genes analyses, exhibited high diversity in all aerosol samples. The following phyla were retrieved: *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Proteobacteria*, and *Firmicutes*, as well as unclassified bacteria. These phyla are usually widespread in nature and were previously found in desert soils [*Janssen*, 2006] as well as aerosols [*Kuske et al.*, 1997; *Katra et al.*, 2014]. Within these phylotypes, the most dominant families (as relative OTUs) were *Pseudomonadaceae* (3–11%), *Bacillaceae* (4–34%), *Rhodobacteraceae* (4–13%), *Cytophagaceae* (3–16%), *Frankiales* (2–8%), and *Chloroflexaceae* (2–8%) (Figure 1b). These retrieved genes are in agreement with
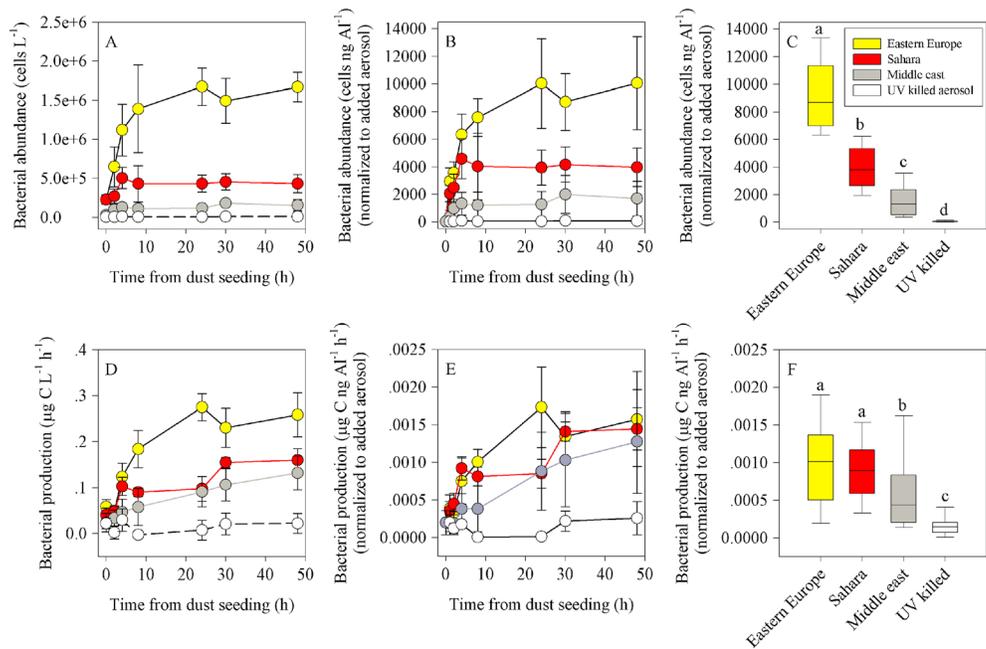
Figure 2. (a–c) Bacterial abundance and (d–f) bacterial production in sterile SEMS water following aerosol collected between 2006 and 2012. Color map is presented in Figure1's legends. (c and f) The box-Whisker plots portion interquartile range from 25th to 75th percentiles. Horizontal bar within the box is the median value. The letters above the box-plots represent significant differences (one-way ANOVA and a Fisher LSD mean comparison test, $P < 0.05$) for mean values between filter types. Data and blank values are presented in Table S3 and Figure S1.

other studies that identified airborne microorganisms in almost every aerosol sample collected worldwide, including the Southern Ocean [*Pósfai et al.*, 2003], the Arctic Ocean [*Leck and Bigg*, 2008], the Middle East [*Griffin et al.*, 2007], the Virgin Island [*Griffin et al.*, 2001], and in freshwater aquatic systems such as mountain lakes in the Alps [*Peter et al.*, 2014] and Mediterranean lake reservoirs [*Reche et al.*, 2009].

Principle component analysis indicated three different clusters of "biological" signatures based on rRNA, which corresponded to the aerosol geographic origin (Figure 1c). Bray-Curtis dissimilarly index showed 24–63% differences between the relative OTUs. Based on this matrix, the Eastern Europe and Sahara aerosols were relatively close (27–36%), whereas the Middle East and Eastern Europe samples exhibited the highest distance from one another (63%) (Table S2). These results highlight the role of biogeography in the diversity of airborne microorganisms that are being delivered into the SEMS via atmospheric depositions.

Data retrieved from field and laboratory controlled experiments, as well as from model simulations, indicate that no simple pattern can explain the high variability usually observed when considering the response of phytoplankton and bacterial abundance and activity to atmospheric deposition [*Guieu et al.*, 2014]. This variability can be attributed to the additions of different types of dust/aerosols, providing nutrients, but also by a wide array of airborne microbes supplied with the dust/aerosols. Thus, the impact of airborne bacteria should also be considered when assessing the biological responses in dust/aerosol addition bioassays. In fact, to date, little is known about the activity of airborne microbes in seawater and how they compete/interact with ambient microbial populations. We hypothesized that due to the unique bacterial assembly in the different aerosol samples (Figure 1), the survival and activity of the airborne microbes from these filters will also vary as different groups have different metabolic needs and growth rates [*Kirchman*, 2012]. We therefore experimentally examined airborne bacterial abundance and activity in sterile surface LNLC SEMS water over the course of 48 h using different aerosol types (i.e., Sahara, Middle East, and Eastern European sources). We assumed that any significant activity above background levels (sterile seawater with or without UV-killed dust) would be a result of the airborne microbes.

Heterotrophic bacterial abundance in the sterile SEMS waters to which aerosols were added gradually increased throughout the experiment duration and especially in the first 8 h following the aerosol addition (Figures 2a and 2b). Bacterial abundance from the aerosol that originated from the Sahara increased

approxiumately twofold 4 h after its addition (compared to the immediate value measured 0.5 h following addition), reaching a plateau after 8 h (Figures 2a and 2b). The Middle East aerosol triggered the same trend, though with a threefold to fourfold increase in airborne bacterial abundance over 48 h (Figures 2a and 2b). Aerosol originating from Eastern Europe exhibited the longest (24 h) and most pronounced (fivefold) increase in bacterial abundance before reaching a plateau (Figures 2a and 2b). Our observations are in agreement with *Peter et al.* [2014] that reported an increase in bacterial abundance following dust deposition in sterile lake waters, however, that was only after 4–5 days and the increase was larger (100-fold). The differences between the studies may result from different airborne bacterial communities, aerosol chemical properties, atmospheric transport processes, and ambient water characteristics. Regardless, the fast and significant ($P < 0.05$) increase of bacterial abundance in sterile seawater following addition of all aerosol types examined here suggests that some airborne bacteria can immediately start interacting with the ambient microbial populations once deposited. This observed increase in bacterial abundance in the sterile seawater corresponds to ~1% of the typical abundance in the SEMS waters [e.g., *Herut et al.*, 2005; *Pulido-Villena et al.*, 2014; *Raveh et al.*, 2015]. Among these airborne microbes, it is possible that only few specialized taxa are viable and can affect the ecology of the receiving environment, and particularly the surface microlayer. It is also possible that the observed increase in bacterial abundance (Figures 2 and S1) could be due to the release of nonactive bacteria alongside viable bacteria. Based on the cell enumeration alone we cannot conclude what fraction of these bacteria were actually viable (active). Further, we cannot rule out that airborne viral introduction has resulted in cell lysis during the 48 h timeframe of the experiment, which pose additional constrains on the bacterial abundance, hence the observed plateau several hours after deposition (Figures 2a and 2b).

The highest airborne bacterial abundances were found in aerosols originating from Eastern Europe and the lowest from the Middle Eastern aerosols (Figure 2c). These differences highlight the fact that the aerosols' origin and their potential route prior to deposition have a significant effect on the number of bacteria contained within their particles as well as on their identity. Similarly, *Griffin et al.* [2002] demonstrated that dust might pick up microorganisms along its route through the adhesion of microbe-laden fine aquatic sprays to dust particles.

While several studies have shown that desert dust contains airborne bacteria (references herein and reviewed by *Griffin* [2007]), and some reports on growth after deposition in freshwater settings exist [*Reche et al.*, 2009; *Peter et al.*, 2014], to our knowledge, no studies reported airborne microbial activity in seawater (i.e., carbon and nitrogen utilization).

Overall, bacterial production increased approximately fourfold relative to time zero (prior to addition) measurements following aerosol additions (Figures 2d, 2e, S1), though with no distinct biogeographical pattern (Figure 2f). Yet cell-specific activity (i.e., bacterial production per bacterial abundance), showed distinct biogeographical trends (Figure S2). These biogeographical differences may be explained by differences in the species of the laden bacterial heterotrophs (Figure 1b), the amounts of nutrient/metal leached from each aerosol type, as well as induced processes during transport due to changes of pH, temperature, and moisture content [*Gans et al.*, 2005; *Janssen*, 2006; *Nenes et al.*, 2011]. The retrieved bacterial production corresponds to ~50% of the typically measured rates in the open SEMS (reviewed in *Pulido-Villena et al.* [2012]) and ~20% of the SEMS coastal water [*Raveh et al.*, 2015]. Thus, in times of high atmospheric deposition, as in the case of the aerosols collected and examined here, airborne bacteria can be important contributors in the SEMS surface (especially in the top microlayer) water's microbial loop.

The bacterial abundance, and more so the activity data, illustrates the potential involvement of airborne microbes in diverse ecological functions in seawater. One possible ecological function of airborne bacteria is exemplified in the N_2 fixation rates, showing that aerosol-associated diazotrophs can become active and fix N_2 (Figure 3). Aerosol addition (collected in February 2015) into sterile SEMS water significantly increased N_2 fixation rates after 24 h incubation ($P < 0.05$; Figure 3). The obtained airborne microbes' net N_2 fixation (0.03 nmol N L^{-1} d^{-1}) are ~16% of the "typically" measured rates in this region (usually <0.2 nmol N L^{-1} d^{-1} [*Bonnet et al.*, 2011; *Yogev et al.*, 2011; *Rahav et al.*, 2013a, 2013b, 2013c]). These results emphasize the potential ecological importance of airborne microbes in LNLC marine environments such as the SEMS, via the involvement in both the carbon and nitrogen cycles. In agreement with our findings, a study from the Gulf of Aqaba reported *nifH* sequences as derived from phylogenetic analyses of aerosol samples associated with Saharan dust [*Foster et al.*, 2009]. Thus, deposited aerosols can potentially stimulate N_2 fixation not only via the addition of limiting nutrients [e.g., *Mills et al.*, 2004; *Moore et al.*, 2009] (see review by *Sohm et al.* [2011]) but also by adding actual diazotrophs
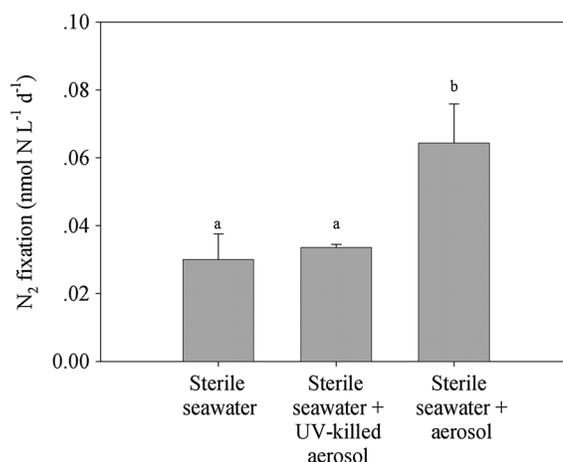
Figure 3. Dinitrogen (N_2) fixation rates following the addition of aerosol (collected during February 2015) to sterile SEMS water. The letters above the columns represent statistically significant differences (one-way ANOVA and a Fisher LSD mean comparison test, $P < 0.05$) for mean values of dinitrogen fixation between treatments.

[*Foster et al.*, 2009]. However, this ecologically important topic requires more research, including the use of molecular tools to identify which airborne diazotroph actually fixes N_2 following deposition (cDNA), under what circumstances, and how common is this phenomenon.

3.2. The Potential Net Impact of Airborne Bacteria in the Mediterranean Sea

The biogeochemical effects of atmospheric deposition on surface LNLC Mediterranean waters were previously assessed using dust/aerosol addition bioassays (in situ microcosms and mesocosms). In all of these bioassay experiments, the net change observed in bacterial abundance or activity and N_2 fixation (whether positive or negative) was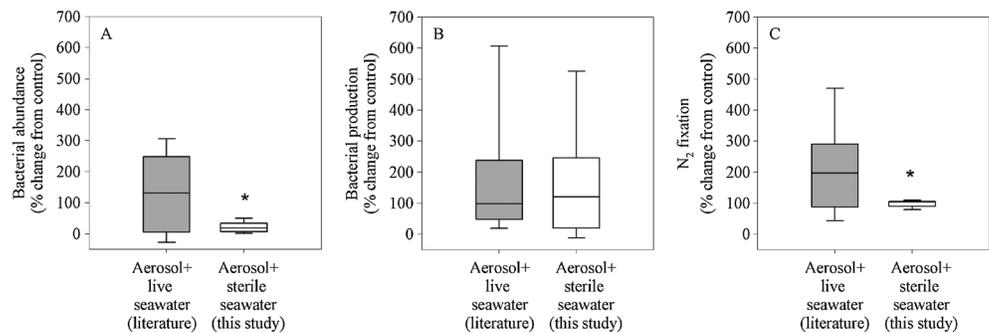 attributed to fertilization by nutrients or trace metals derived from the added dust/aerosol. Yet all of these studies overlooked the possible impact of the added airborne bacteria along with the leached nutrients. We compiled the percent change ($T_{final}/T_{control} \times 100$) in bacterial abundance, bacterial production, and dinitrogen fixation rates from all of these bioassays [*Herut et al.*, 2005; *Romero et al.*, 2011; *Ridame et al.*, 2011; 2013; *Ternon et al.*, 2011; *Pulido-Villena et al.*, 2008, 2014] (Pitta et al., unpublished) and compared them to the net change attributed to airborne microbes as measured in this study (Figure 4). This comparison allowed us to estimate the potential contribution of airborne microbes to the total observed effects of dust/aerosol addition bioassays in the LNLC Mediterranean waters (i.e., both chemical and biological effects). The percentage contribution of heterotrophic airborne bacterial abundance was one third (~33%) of the average change observed following similar aerosol amendments as compiled from the above experimental studies in the Mediterranean Sea (Figure 4a). This emphasizes the significant contribution of airborne bacteria to the observed change in these bioassay experiments. The percent change in bacterial production rates following aerosol additions were similar in both the above reported studies and in this study, emphasizing equal contribution of the chemical and biological components (Figure 4b). The different relative contributions of bacterial abundance and production suggest that the airborne microbes exhibit relative higher activity per cell (i.e., low abundance and high production rates). Furthermore, the airborne dinitrogen fixation contributed ~50% of the total change following aerosol amendments in surface Mediterranean seawater (Figure 4c), suggesting that atmospheric depositions can inject new viable diazotrophs, along with dissolved nutrients.

4. Conclusions

Climate change and desertification may increase dust storm events and frequencies in vast areas of the ocean [*Prospero and Lamb*, 2003; *Guieu et al.*, 2014]. Several studies have shown that these dust/aerosol particles can act as a long-range mobilization vector for microorganisms [e.g., *Kellogg and Griffin*, 2006; *Griffin*, 2007], including of rare bacterial taxa [e.g., *Peter et al.*, 2014]. Yet to date, very little is known about the potential impacts of airborne microbes upon deposition into marine ecosystems and about their influence on biogeochemical processes. Further, the mechanisms that enable airborne bacterial populations to cope with the ambient microbial populations, and possibly outcompete them, are currently vague. It is possible that airborne bacteria are fast utilizers of organic carbon and inorganic nutrients, have toxic/allelopathic/pathogenic functions, or that they can synergistically cooccur with ambient microbial populations. We address here some of these issues demonstrating that aerosols from different geographical sources (Figure 1a) can carry unique consortia of heterotrophic microorganisms (Figures 1b and 1c). We assume that the harsh environmental conditions during transport likely act as an environmental filter, thus only specialized taxa, possibly with

Figure 4. The percent change ($T_{final}/T_{control}$) of (a) bacterial abundance (BA), (b) bacterial production (BP), and (c) N_2 fixation in bioassays aerosol additions to live (ambient, nonkilled) surface seawater from the oligotrophic Mediterranean Sea [*Herut et al.*, 2005; *Romero et al.*, 2011; *Ridame et al.*, 2011; 2013; *Ternon et al.*, 2011; *Pulido-Villena et al.*, 2008, 2014] (*Pitta et al.*, unpublished) and to sterile SEMS water (this study). The percent change is normalized to 1.5 mg L^{-1} aerosol addition; the asterisk represents significant statistical difference ($\alpha = 0.05$).

protecting mechanisms such as high DNA GC content [*Matallana-Surget et al.*, 2008], sporulation [*Jones and Lennon*, 2010], or accumulation of photo-protecting pigments [*Tong and Lighthart*, 1997], can remain viable. Thus, biogeographical aspects are important controlling factors that will possibly introduce new system functions and should be studied both spatially and temporally. This might be especially important in the SEMS, where increase in atmospheric deposition intensity and frequency is expected [*Rogora et al.*, 2004; *Guieu et al.*, 2014]. Our results also demonstrate that some of the airborne microorganisms exhibit high cell-specific activity (Figure S2), allowing interactions with ambient microbial populations immediately upon deposition. Furthermore, we show that airborne bacteria are active, fixing both carbon (Figure 2), and nitrogen (Figure 3). Dinitrogen fixation mediate by airborne microbes can contribute to the current debate on factors controlling this process in the Mediterranean Sea [*Berman-Frank and Rahav*, 2012], as well as to the biogeochemical role of atmospheric deposition in LNLC regions [e.g., *Sohm et al.*, 2011].

Finally, desert dust/aerosols usually carry small-size autotrophic cyanobacteria, fungi, and viruses [*Griffin*, 2007; *Lang-Yona et al.*, 2014; *Sharoni et al.*, 2015]. These organisms may have additional ecological implications in the ocean [*Vardi et al.*, 2012], including on microbial composition [*Suttle*, 2007], or may affect marine organisms via pathogenesis [*Sharoni et al.*, 2015].

Taken together, dust/aerosols dispersal can be a potential source of a wide array of organisms, which may impact microbial composition and food web dynamics in marine environments. These potential ecological roles are just beginning to be recognized [*Smith*, 2013] and should be taken into account in future studies when estimating the impact of dust in surface marine environments.

References

Azam, F., and F. Malfatti (2007), Microbial structuring of marine ecosystems, *Nat. Rev. Microbiol.*, 5(10), 782–791, doi:10.1038/nrmicro1747.

Berman-Frank, I., and E. Rahav (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*, pp. 199–226, Nova Science Publishers, New York.

Bonnet, S., O. Grosso, and T. Moutin (2011), Planktonic dinitrogen fixation in the Mediterranean Sea: A major biogeochemical process during the stratified period?, *Biogeosciences*, 8(1), 1197–1225, doi:10.5194/bgd-8-1197-2011.

Bosc, E., A. Bricaud, and D. Antoine (2004), Seasonal and interannual variability in algal biomass and primary production in the Mediterranean Sea, as derived from 4 years of SeaWiFS observations, *Global Biogeochem. Cycles*, 18, GB1005, doi:10.1029/2003GB002034.

Buchan, A., G. R. LeCleir, C. A. Gulvik, and J. M. González (2014), Master recyclers: Features and functions of bacteria associated with phytoplankton blooms, *Nat. Rev. Microbiol.*, 12, 686–698, doi:10.1038/nrmicro3326.

Burrows, S. M., W. Elbert, M. G. Lawrence, and U. Pöschl (2009), Bacteria in the global atmosphere – Part 1: Review and synthesis of literature data for different ecosystems, *Atmos. Chem. Phys.*, 9(23), 9263–9280, doi:10.5194/acp-9-9263-2009.

Caporaso, J. G., J. Kuczynski, and J. Stombaugh (2010), QIIME allows analysis of high-throughput community sequencing data, *Nat. Methods*, 7(5), 335–336.

Cullen, J. J., and P. W. Boyd (2008), Predicting and verifying the intended and unintended consequences of large-scale ocean iron fertilization, *Mar. Ecol. Prog. Ser.*, 364, 295–301, doi:10.3354/meps07551.

Dayan, U., J. Heffter, J. Miller, and G. Gutman (1991), Dust intrusion events into the Mediterranean Basin, *Am. Meteorol. Soc.*, 30, 1185–1199.

Foster, R. A., A. Paytan, and J. P. Zehr (2009), Seasonality of N$_2$ fixation and *nifH* gene diversity in the Gulf of Aqaba (Red Sea), *Limnol. Oceanogr.*, 54(1), 219–233, doi:10.4319/lo.2009.54.1.0219.

Ganor, E., I. Osetinsky, A. Stupp, and P. Alpert (2010), Increasing trend of african dust, over 49 years, in the eastern Mediterranean, *J. Geophys. Res.*, 115(7), 1–7, doi:10.1029/2009JD012500.

Gans, J., M. Wolinsky, and J. Dunbar (2005), Computational improvements reveal great bacterial diversity and high metal toxicity in soil, *Science*, *309*(5739), 1387–1390, doi:10.1126/science.1112665.

Griffin, D. W. (2007), Atmospheric movement of microorganisms in clouds of desert dust and implications for human health, *Clin. Microbiol. Rev.*, *20*(3), 459–477, doi:10.1128/CMR.00039-06.

Griffin, D. W., V. H. Garrison, J. R. Herman, and E. A. Shinn (2001), African desert dust in the Caribbean atmosphere: Microbiology and public health, *Aerobiologia*, *17*, 203–213.

Griffin, D. W., C. A. Kellogg, V. H. Garrison, and E. A. Shinn (2002), The global transport of dust, *Am. Sci.*, *90*, 228–235.

Griffin, D. W., N. Kubilay, M. Koçak, M. A. Gray, T. C. Borden, and E. A. Shinn (2007), Airborne desert dust and aeromicrobiology over the Turkish Mediterranean coastline, *Atmos. Environ.*, *41*(19), 4050–4062, doi:10.1016/j.atmosenv.2007.01.023.

Gruber, N., et al. (2011), Warming up, turning sour, losing breath: An integrated observing system for ocean biogeochemistry at a time of change, *R. Soc.*, *369*(1943), 1980–1996.

Guerzoni, S., et al. (1999), The role of atmospheric deposition in the biogeochemistry of the Mediterranean Sea, *Prog. Oceanogr.*, *44*(1–3), 147–190, doi:10.1016/S0079-6611(99)00024-5.

Guieu, C., et al. (2014), Global biogeochemical cycles deposition to low nutrient low chlorophyll regions, *Global Biogeochem. Cycles*, *28*, 1179–1198, doi:10.1002/2014GB004852.

Herut, B., M. D. Krom, G. Pan, and R. Mortimer (1999), Atmospheric input of nitrogen and phosphorus to the southeast Mediterranean: Sources, fluxes, and possible impact, *Limnol. Oceanogr.*, *44*(7), 1683–1692, doi:10.4319/lo.1999.44.7.1683.

Herut, B., R. Collier, and M. D. Krom (2002), The role of dust in supplying nitrogen and phosphorus to the southeast Mediterranean, *Limnol. Oceanogr.*, *47*(3), 870–878, doi:10.4319/lo.2002.47.3.0870.

Herut, B., T. Zohary, M. D. Krom, R. F. C. Mantoura, P. Pitta, S. Psarra, F. Rassoulzadegan, T. Tanaka, and T. F. Thingstad (2005), Response of east Mediterranean surface water to Saharan dust: On-board microcosm experiment and field observations, *Deep Sea Res., Part II*, *52*(22–23), 3024–3040, doi:10.1016/j.dsr2.2005.09.003.

Janssen, P. H. (2006), Identifying the dominant soil bacterial taxa in libraries of 16S rRNA and 16S rRNA genes, *Appl. Environ. Microbiol.*, *72*(3), 1719–1728, doi:10.1128/AEM.72.3.1719.

Jones, S. E., and J. T. Lennon (2010), Dormancy contributes to the maintenance of microbial diversity, *Proc. Natl. Acad. Sci. U.S.A.*, *107*(13), 5881–5886, doi:10.1073/pnas.0912765107.

Jordi, A., G. Basterretxea, A. Tovar-Sánchez, A. Alastuey, and X. Querol (2012), Copper aerosols inhibit phytoplankton growth in the Mediterranean Sea, *Proc. Natl. Acad. Sci. U.S.A.*, *109*(52), 21,246–21,249, doi:10.1073/pnas.1207567110.

Katra, I., L. Arotsker, H. Krasnov, A. Zaritsky, A. Kushmaro, and E. Ben-Dov (2014), Richness and diversity in dust storm borne biomes at the southeast Mediterranean, *Sci. Rep.*, *4*, 5265, doi:10.1038/srep05265.

Kellogg, C. A., and D. W. Griffin (2006), Aerobiology and the global transport of desert dust, *Trends Ecol. Evol.*, *21*(11), 638–644, doi:10.1016/j.tree.2006.07.004.

Kirchman, D. L. (2012), in *Processes in Microbial Ecology*, edited by D. L. Kirchman, 1st ed., pp., Oxford Univ. Press, Oxford.

Koçak, M., N. Kubilay, and N. Mihalopoulos (2004), Ionic composition of lower tropospheric aerosols at a Northeastern Mediterranean site: Implications regarding sources and long-range transport, *Atmos. Environ.*, *38*(14), 2067–2077.

Koçak, M., N. Kubilay, B. Herut, and M. Nimmo (2005), Dry atmospheric fluxes of trace metals (Al, Fe, Mn, Pb, Cd, Zn, Cu) over the Levantine Basin: A refined assessment, *Atmos. Environ.*, *39*(38), 7330–7341, doi:10.1016/j.atmosenv.2005.09.010.

Koçak, M., N. Kubilay, S. Tuğrul, and N. Mihalopoulos (2010), Atmospheric nutrient inputs to the northern Levantine basin from a long-term observation: Sources and comparison with riverine inputs, *Biogeosciences*, *7*(12), 4037–4050, doi:10.5194/bg-7-4037-2010.

Kress, N., and B. Herut (2001), Spatial and seasonal evolution of dissolved oxygen and nutrients in the southern Levantine Basin (eastern Mediterranean Sea): chemical characterization of the water masses and inferences on the N: P ratios, *Deep Sea Res. Part I*, *48*(11), 2347–2372.

Kress, N., I. Gertman, and B. Herut (2014), Temporal evolution of physical and chemical characteristics of the water column in the easternmost Levantine Basin (eastern Mediterranean Sea) from 2002 to 2010, *J. Mar. Syst.*, *135*, 6–13.

Krom, M. D., N. Kress, S. Brenner, and L. I. Gordon (1991), Phosphorus limitation of primary productivity in the eastern Mediterranean Sea, *Limnol. Oceanogr.*, *36*(3), 424–432, doi:10.4319/lo.1991.36.3.0424.

Kuske, C. R., S. M. Barns, and J. D. Busch (1997), Diverse uncultivated bacterial groups from soils of the arid southwestern United States that are present in many geographic regions, *Appl. Environ. Microbiol.*, *63*(9), 3614–3621.

Langille, M. G. I., et al. (2013), Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences, *Nat. Biotechnol.*, *31*(9), 814–821, doi:10.1038/nbt.2676.

Lang-Yona, N., Y. Lehahn, B. Herut, N. Burshtein, and Y. Rudich (2014), Marine aerosol as a possible source for endotoxins in coastal areas, *Sci. Total Environ.*, *499*, 311–318, doi:10.1016/j.scitotenv.2014.08.054.

Lawrence, C. R., and J. C. Neff (2009), The contemporary physical and chemical flux of aeolian dust: A synthesis of direct measurements of dust deposition, *Chem. Geol.*, *267*(1–2), 46–63, doi:10.1016/j.chemgeo.2009.02.005.

Leck, C., and E. K. Bigg (2008), Comparison of sources and nature of the tropical aerosol with the summer high Arctic aerosol, *Tellus, Ser. B*, *60B*(1), 118–126, doi:10.1111/j.1600-0889.2007.00315.x.

Léon, J.-F., P. Augustin, M. Mallet, T. Bourrianne, F. Dulac, M. Fourmentin, D. Lambert, and B. Sauvage (2015), Aerosol vertical distribution, optical properties and transport over Corsica (western Mediterranean), *Atmos. Chem. Phys. Discuss.*, *15*(6), 9507–9540, doi:10.5194/acpd-15-9507-2015.

Mackey, K. R. M., K. Roberts, M. W. Lomas, M. A. Saito, A. F. Post, and A. Paytan (2012), Enhanced solubility and ecological impact of atmospheric phosphorus deposition upon extended seawater exposure, *Environ. Sci. Technol.*, *46*(19), 10,438–10,446, doi:10.1021/es3007996.

Mahowald, N. M. (2007), Anthropocene changes in desert area: Sensitivity to climate model predictions, *Geophys. Res. Lett.*, *34*, L18817, doi:10.1029/2007GL030472.

Massana, R., A. E. Murray, C. M. Preston, and E. Delong (1997), Vertical distribution and phylogenetic characterization of marine planktonic Archaea in the Santa Barbara Channel, *Appl. Environ. Microbiol.*, *63*(1), 50–56.

Matallana-Surget, S., J. A. Meador, F. Joux, and T. Douki (2008), Effect of the GC content of DNA on the distribution of UVB-induced bipyrimidine photoproducts, *Photochem. Photobiol. Sci.*, *7*, 794–801, doi:10.1039/B719929E.

Mills, M. M., C. Ridame, M. Davey, J. LaRoche, and R. J. Geider (2004), Iron and phosphorus co-limit nitrogen fixation in the eastern tropical North Atlantic, *Nature*, *429*(6989), 292–4, doi:10.1038/nature02550.

Mohr, W., T. Großkopf, D. W. R. Wallace, and J. LaRoche (2010), Methodological underestimation of oceanic nitrogen fixation rates, *PLoS One*, *5*(9), 1–7, doi:10.1371/journal.pone.0012583.

Moore, M. C., et al. (2009), Large-scale distribution of Atlantic nitrogen fixation controlled by iron availability, *Nat. Geosci.*, *2*(12), 867–871, doi:10.1038/ngeo667.

Neff, J. C., A. P. Ballantyne, G. L. Farmer, N. M. Mahowald, J. L. Conroy, C. C. Landry, J. T. Overpeck, T. H. Painter, C. R. Lawrence, and R. L. Reynolds (2008), Increasing eolian dust deposition in the western United States linked to human activity, *Nat. Geosci.*, *1*, 189–195, doi:10.1038/ngeo133.

Nenes, A., M. D. Krom, N. Mihalopoulos, P. Van-Cappellen, Z. Shi, A. Bougiatioti, P. Zarmpas, and B. Herut (2011), Atmospheric acidification of mineral aerosols: A source of bioavailable phosphorus for the oceans, *Atmos. Chem. Phys. (ACP)*, *11*, 6265–6272.

Paytan, A., K. R. M. Mackey, Y. Chen, I. D. Lima, S. C. Doney, N. Mahowald, R. Labiosa, and A. F. Post (2009), Toxicity of atmospheric aerosols on marine phytoplankton, *Proc. Natl. Acad. Sci. U.S.A.*, *106*(12), 4601–4605, doi:10.1073/pnas.0811486106.

Perkins, S. (2001), Dust, the thermostat, *Sci. News*, *160*(13), 200–202, doi:10.2307/4012776.

Peter, H., P. Hörtnagl, I. Reche, and R. Sommaruga (2014), Bacterial diversity and composition during rain eventswith and without Saharan dust influence reaching a high mountain lake in the Alps, *Environ. Microbiol. Rep.*, *6*(6), 618–624, doi:10.1111/1758-2229.12175.

Polovina, J. J., E. A. Howell, and M. Abecassis (2008), Ocean's least productive waters are expanding, *Geophys. Res. Lett.*, *35*, L03618, doi:10.1029/2007GL031745.

Polymenakou, P. N. (2012), Atmosphere: A source of pathogenic or beneficial microbes?, *Atmosphere*, *3*(1), 87–102, doi:10.3390/atmos3010087.

Pósfai, M., J. Li, J. R. Anderson, and P. R. Buseck (2003), Aerosol bacteria over the southern Ocean during ACE-1, *Atmos. Res.*, *66*(4), 231–240, doi:10.1016/S0169-8095(03)00039-5.

Prospero, J. M., and P. J. Lamb (2003), African droughts and dust transport to the Caribbean: Climate change implications, *Science*, doi:10.1126/science.1089915.

Pulido-Villena, E., T. Wagener, and C. Guieu (2008), Bacterial response to dust pulses in the western Mediterranean: Implications for carbon cycling in the oligotrophic ocean, *Global Biogeochem. Cycles*, *22*, GB1020, doi:10.1029/2007GB003091.

Pulido-Villena, E., J. F. Ghiglione, E. Ortega-Retuerta, F. Van Wambeke, and T. Zohary (2012), Heterotrophic bacteria in the pelagic realm of the Mediterranean Sea, in Life in the Mediterranean Sea: A look at habitat changes, edited by N. Stambler, pp. 227–265, Nova Science Publishers, New York.

Pulido-Villena, E., A.-C. Baudoux, I. Obernosterer, M. Landa, J. Caparros, P. Catala, C. Georges, J. Harmand, and C. Guieu (2014), Microbial food web dynamics in response to a Saharan dust event: Results from a mesocosm study in the oligotrophic Mediterranean Sea, *Biogeosciences*, *11*(1), 337–371, doi:10.5194/bgd-11-337-2014.

Rahav, E., B. Herut, A. Levi, M. R. Mulholland, and I. Berman-Frank (2013a), Springtime contribution of dinitrogen fixation to primary production across the Mediterranean Sea, *Ocean Sci.*, *9*(3), 489–498.

Rahav, E., E. Bar-Zeev, S. Ohayon, H. Elifantz, N. Belkin, B. Herut, M. R. Mulholland, and I. Berman-Frank (2013b), Dinitrogen fixation in aphotic oxygenated marine environments, *Front. Microbiol.*, *4*, 227, doi:10.3389/fmicb.2013.00227.

Rahav, E., B. Herut, N. Stambler, E. Bar-Zeev, M. R. Mulholland, and I. Berman-Frank (2013c), Uncoupling between dinitrogen fixation and primary productivity in the eastern Mediterranean Sea, *J. Geophys. Res. Biogeosci.*, *118*, 195–202, doi:10.1002/jgrg.20023.

Raveh, O., N. David, G. Rilov, and E. Rahav (2015), The temporal dynamics of coastal phytoplankton and bacterioplankton in the Eastern Mediterranean Sea, *Plos One*, *10*(10), e0140690, doi:10.1371/journal.pone.0140690.

Reche, I., E. Ortega-Retuerta, O. Romera, E. Pulido-Villena, R. Morales-Baquero, and E. O. Casamayor (2009), Effect of Saharan dust inputs on bacterial activity and community composition in Mediterranean lakes and reservoirs, *Limnol. Oceanogr.*, *54*(3), 869–879, doi:10.4319/lo.2009.54.3.0869.

Ridame, C., M. Le Moal, C. Guieu, E. Ternon, I. C. Biegala, S. L'Helguen, and M. Pujo-Pay (2011), Nutrient control of N_2 fixation in the oligotrophic Mediterranean Sea and the impact of Saharan dust events, *Biogeosciences*, *8*, 2773–2783, doi:10.5194/bg-8-2773-2011.

Ridame, C., C. Guieu, and S. L'Helguen (2013), Strong stimulation of N_2 fixation in oligotrophic Mediterranean Sea: Results from dust addition in large in situ mesocosms, *Biogeosciences*, *10*(11), 7333–7346, doi:10.5194/bg-10-7333-2013.

Rogora, M., R. Mosello, and A. Marchetto (2004), Long-term trends in the chemistry of atmospheric deposition in Northwestern Italy: The role of increasing Saharan dust deposition, *Tellus B*, *56*(5), 426–434, doi:10.1111/j.1600-0889.2004.00114.x.

Romero, E., F. Peters, C. Marrasé, S. Guadayol, J. M. Gasol, and M. G. Weinbauer (2011), Coastal Mediterranean plankton stimulation dynamics through a dust storm event: An experimental simulation, *Estuarine Coastal Shelf Sci.*, *93*(1), 27–39, doi:10.1016/j.ecss.2011.03.019.

Sharoni, S., et al. (2015), Infection of phytoplankton by aerosolized marine viruses, *Proc. Natl. Acad. Sci. U.S.A.*, *112*(21), 6643–6647, doi:10.1073/pnas.1423667112.

Simon, M., A. Alldredge, and F. Azam (1989), Protein-content and protein-synthesis rates of planktonic marine-bacteria, *Mar. Ecol. Prog. Ser.*, *51*(3), 201–213.

Simon, M., A. Alldredge, and F. Azam (1990), Bacterial carbon dynamics on marine snow, *Mar. Ecol. Prog. Ser.*, *65*(3), 205–211.

Smith, D. J. (2013), Aeroplankton and the need for a global monitoring network, *BioScience*, *63*(7), 515–516, doi:10.1525/bio.2013.63.7.3.

Sohm, J. A., E. A. Webb, and D. G. Capone (2011), Emerging patterns of marine nitrogen fixation, *Nat. Rev. Microbiol.*, *9*(7), 499–508, doi:10.1038/nrmicro2594.

Steinacher, M., F. Joos, T. L. Frölicher, L. Bopp, P. Cadule, S. C. Doney, M. Gehlen, B. Schneider, and J. Segschneider (2010), Projected 21st century decrease in marine productivity: A multi-model analysis, *Biogeosciences*, *6*(4), 7933–7981, doi:10.5194/bgd-6-7933-2009.

Suttle, C. A. (2007), Marine viruses - Major players in the global ecosystem, *Nat. Rev. Microbiol.*, *5*, 801–812, doi:10.1038/nrmicro1750.

Ternon, E., C. Guieu, C. Ridame, S. L'Helguen, and P. Catala (2011), Longitudinal variability of the biogeochemical role of Mediterranean aerosols in the Mediterranean Sea, *Biogeosciences*, *8*(5), 1067–1080, doi:10.5194/bg-8-1067-2011.

Tong, Y. Y., and B. Lighthart (1997), Solar radiation is shown to select for pigmented bacteria in the ambient outdoor atmosphere, *Photochem. Photobiol.*, *65*(65), 103–106, doi:10.1111/j.1751-1097.1997.tb01884.x.

Uitz, J., H. Claustre, B. Gentili, and D. Stramski (2010), Phytoplankton class-specific primary production in the world's oceans: Seasonal and interannual variability from satellite observations, *Global Biogeochem. Cycles*, *24*, GB3016, doi:10.1029/2009GB003680.

Vardi, A., L. Haramaty, B. A. S. Van Mooy, H. F. Fredricks, S. A. Kimmance, A. Larsen, and K. D. Bidle (2012), Host-virus dynamics and subcellular controls of cell fate in a natural coccolithophore population, *Proc. Natl. Acad. Sci. U.S.A.*, *109*(47), 19,327–19,332, doi:10.1073/pnas.1208895109.

Vaulot, D., and D. Marie (1999), Diel variability of photosynthetic picoplankton in the equatorial Pacific, *Appl. Environ. Microbiol.*, *104*(C2), 3297–3310.

Womack, A. M., B. J. M. Bohannan, and J. L. Green (2010), Biodiversity and biogeography of the atmosphere, *Philos. Trans. R. Soc., B*, *365*(1558), 3645–3653, doi:10.1098/rstb.2010.0283.

Yogev, T., et al. (2011), Is dinitrogen fixation significant in the Levantine Basin, East Mediterranean Sea?, *Environ. Microbiol.*, *13*(4), 854–871.