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Research Article

RESEARCH ARTICLE

Dust-borne microbes affect *Ulva ohnoi*'s growth and physiological state

Nimrod Krupnik[1,2,*], Dorin Theodora Asis[3], Natalia Belkin[1], Maxim Rubin-Blum[1,†], Álvaro Israel[1], Adina Paytan[4], David Meiri[2], Barak Herut[1] and Eyal Rahav[1,*,‡]

[1]Israel Oceanographic and Limnological Research, Tel-Shikmona 8030, Haifa, 310800, Israel, [2]Department of Biology, Technion-Israel Institute of Technology, Technion City, Haifa, 3200003, Israel, [3]Department of Evolutionary and Human Biology, University of Haifa, Abba Khoushy Ave 199, Haifa, 3498838, Israel and [4]Institute of Marine Science, University of California,1156 High St, Santa Cruz, CA, 95064, USA

*Corresponding authors: Biology Department, Israel Oceanographic & Limnological Research, Tel-Shikmona 8030, Haifa, 310800, Israel. Tel: +972-48565260; E-mails: nimrodkru@campus.technion.ac.il; eyal.rahav@ocean.org.il

One sentence summary: Viable airborne microbes can restore *U. ohnoi* natural microbial epibionts communities, thereby keeping the seaweed alive and 'healthy'.

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†Maxim Rubin-Blum, http://orcid.org/0000-0003-2953-3161
‡Eyal Rahav, http://orcid.org/0000-0001-5697-6684

ABSTRACT

The marine macroalgae *Ulva* sp. is considered an ecosystem engineer in rocky shores of temperate waters worldwide. *Ulva* sp. harbors a rich diversity of associated microbial epibionts, which are known to affect the algae's typical morphological development and 'health'. We examined the interaction between airborne microbes derived from atmospheric aerosols and *Ulva ohnoi* growth and physiological state. Specifically, we measured *U. ohnoi* growth rates and photosynthetic efficiency (*Fv/Fm*), alongside its microbial epibionts abundance, activity and diversity following dust (containing nutrients and airborne microorganisms) or UV-treated dust (only nutrients) amendments to filtered seawater. Parallel incubations with epibionts-free *U. ohnoi* (treated with antibiotics that removed the algae epibionts) were also tested to specifically examine if dust-borne microbes can replenish the epibiont community of *U. ohnoi*. We show that viable airborne microbes can restore *U. ohnoi* natural microbial epibionts communities, thereby keeping the seaweed alive and 'healthy'. These results suggest that microbes delivered through atmospheric aerosols can affect epiphyte biodiversity in marine flora, especially in areas subjected to high annual atmospheric dust deposition such as the Mediterranean Sea.

Keywords: *Ulva* sp.; airborne microbes; bacterial abundance; bacterial production; *Fv/Fm*; daily growth rate

INTRODUCTION

Marine macroalgae play a pivotal role in the ecology of coastal environments (Schiel and Foster 2006; Krupnik *et al.* 2018) and are considered to be ecosystem engineers (Egan, Thomas and Kjelleberg 2008; Egan *et al.* 2013). Macroalgae are important primary producers (Krause-Jensen and Duarte 2016) and support ecological niches for many other marine organisms (Haywood, Vance and Loneragan 1995; Bulleri *et al.* 2002). Macroalgae have a tight relationship with diverse populations of epiphytic microbial symbionts that are crucial for the wellbeing of their hosts (Beleneva and Zhukova 2006; Burke *et al.* 2011; Wahl *et al.* 2012). Some macroalgae species develop a wild-type phenotype only

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when their microbial epibionts are present (Provasoli and Pintner 1980; Nakanishi *et al*. 1996; Matsuo *et al*. 2003; Marshall *et al*. 2006). It has further been shown that microbial epibiont communities on the seaweed surface may induce the release and settlement of algal spores (Joint, Tait and Wheeler 2007; Weinberger *et al*. 2007), affect growth (Keshtacher-Liebson, Hadar and Chen 1995; Croft, Warren and Smith 2006) and induce rhizoid formation (Ghaderiardakani, Coates and Wichard 2017). The nature of the association between the macroalgae and their epibionts is still unclear. While the epiphytic microbes can be specific to the algae species (Lachnit *et al*. 2009, 2011), the competitive lottery model claims that distinct microbial communities with similar eco-physiological characteristics can thrive on the same algal species, hence the interaction is not specific to a certain microbial lineage but rather to its functionality and ecological role (Burke *et al*. 2011). In both cases, the interactions between the algae and the epibionts result in obligatory symbioses (Egan *et al*. 2013).

The microbial epiphytic communities of macroalgae are diverse and often comprise proteobacterial lineages (Rhodobacteraceae, Sphingomonadaceae and unclassified Gammaproteobacteria families), Bacteroidota (Flavobacteriaceae, Bacteroidaceae—Bacteroidetes) and Planctomycetaceae (Longford *et al*. 2007; Hempel *et al*. 2008; Staufenberger *et al*. 2008; Tujula *et al*. 2010; Burke *et al*. 2011). The composition of these communities usually differs from that of the surrounding seawater (Staufenberger *et al*. 2008; Burke *et al*. 2011), highlighting the strong association between these microbes and their macroalgae host. Yet, the current knowledge on macroalgae's epibionts, in particular, their biodiversity, physiology and interaction with the surrounding biota, is scarce, and little is known about how the macroalgae acquire their symbionts (Burke *et al*. 2011).

Aerosols and dust are transported across the oceans and were recognized as an important source of micro and macronutrients (Herut, Collier and Krom 2002; Jickells *et al*. 2005; Duce *et al*. 2008), thus affecting algae biomass and primary production (Mackey *et al*. 2012; Chien *et al*. 2016; Rahav *et al*. 2018a). In addition, aerosols and dust contain airborne microbes (Griffin 2007; Reche *et al*. 2009; Rahav *et al*. 2016c, 2016a), some of which are known macroalgae epibionts, including Flavobacteriaceae and Bacteroidaceae. Airborne microorganisms may remain viable during atmospheric transport (Womack, Bohannan and Green. 2010; Polymenakou 2012; Rahav *et al*. 2019a) becoming active upon deposition to seawater (Rahav et al. 2016b, 2016c; Mescioglu *et al*. 2019b) or freshwater (Reche *et al*. 2009; Peter *et al*. 2014) bodies. Viable airborne microbes were shown to fix bicarbonate and dinitrogen, produce phytoplankton/bacterial metabolites, and express genes needed for antibiotic resistance (Reche *et al*. 2009; Peter *et al*. 2014; Rahav *et al*. 2016b, 2018b; Li *et al*. 2018). To the best of our knowledge, very few studies tested if and how the microbial communities transported by desert dust affect downwind coastal ecosystems with *Ulva* turfs. Here, we experimentally investigated the impact of airborne microbes associated with dust collected from the Saharan Desert on the epibionts and general physiology of the marine green macroalgae *Ulva ohnoi*. We hypothesized that viable airborne microbes can replace *U. ohnoi* natural microbial epibiont communities, consistent with the 'competitive lottery' theory (Egan *et al*. 2013).

MATERIAL AND METHODS

Experimental design

Ulva ohnoi specimens were collected from an outdoor 1 m^3 tank with seawater flow-through to maintain ambient temperature, light and nutrient levels at the Israel Oceanographic & Limnological Research (IOLR), Haifa, Israel (Israel, Golberg and Neori 2019; www.seaweedherbarium.com). Leaf-like individuals (9 cm^2, ~300 mg fresh-weight) were placed in prewashed (10% hydrochloric-acid and rinsed three times with Milli-Q water) Erlenmeyer beakers (500 ml, Pyrex, 4 specimens in each) and acclimated under controlled temperature (20°C) and light (~100 μmol m^{-2} s^{-1} of white fluorescent irradiance, 12:12 light: dark cycle) conditions in 0.2 μm filtered seawater for 2 weeks. After acclimation, six dust-addition microcosms were made in biological triplicates (Fig. 1): [1] Control—no addition; [2] 1.5 mg L^{-1} Saharan dust (SD); [3] 100 μl L^{-1} of a penicillin-streptomycin solution (Sigma P4333) (AB); [4] AB+SD; [5] AB+ 1.5 mg L^{-1} ultra-violet (UV)-treated SD and [6] UV-treated SD (see more details for rational in Fig. 1). For each set of treatments, a single Ulva ohnoi specimen was used per replicate. The amount of dust added (1.5 mg L^{-1}) was within the range of natural atmospheric deposition to the upper mixed layer of the Eastern Mediterranean Sea (~10 m) during intense dust storm events (Herut *et al*. 2005) and aligned with previous studies examining the role of dust on microbes in this system (Astrahan *et al*. 2016; Herut *et al*. 2016; Krom *et al*. 2016; Tsagaraki *et al*.2017). Specimens of *U. ohnoi* were collected from the Erlenmeyer flasks and analyzed for bacterial abundance, bacterial production, photosynthetic efficiency (*Fv/Fm*), algal growth rate and epibiont's microbial diversity following 1-week incubation. AB was added to the relevant Erlenmeyer flasks containing *U. ohnoi* specimens for 5 h. The relevant specimens were then washed 3 times with filtered seawater (FSW) to remove any AB residuals, and transferred to new Erlenmeyer flasks with FSW. The antibiotic treatment reduced *Ulva* epibionts abundance and activity by 80% and 93%, respectively (Fig. S1, Supporting Information). Penicillin-streptomycin is a wide-range antibiotic solution that affects both gram-positive and gram-negative bacteria. As the marine environment hosts both bacterial groups (Anwar and Choi 2014), penicillin-streptomycin was used to pretreat the *Ulva* in experiments 3 (AB), 4 (AB+SD) and 5 (AB + UV-treated SD) in order to de-activate prokaryotic production and effectively kill the majority of the microbial epibiont cells (Das, Mukherjee and Sen 2008, Fig. S1, Supporting Information). The concentration of penicillin-streptomycin used here is the recommended averaged inhibitory concentration used to de-activate many different Gram-positive and Gram-negative bacterial strains (Das, Mukherjee and Sen 2008).

Aerosols were collected during an intense dust storm on February 15th, 2015 at the Eastern Mediterranean coast (latitude 32.28°N, longitude 34.95°E) using a glass plate (for more details on the dust's chemical characteristics see Astrahan *et al*. 2016). Dust particles were transferred to sterile tubes and kept frozen at −20°C until the experiment was carried out in June 2018. Backward trajectory analysis using the HYSPLIT model shows that the collected dust had originated from the Sahara Desert (Fig. S2, Supporting Information).

Microscopy analysis of Ulva ohnoi tissue

Horizontal cross-sections of *U. ohnoi* tissue (~0.25 cm^2) were photographed with an Olympus BH-2 upright microscope equipped with a DFK 41BF02 camera using X20 and X40 magnifications following 1 week of incubation.

Daily Growth Rate (DGR)

Growth rates were estimated from measurements of *U. ohnoi* fresh-weight at the beginning (W_0) and at the end (Wt) of the incubation period (T = 1 week), and expressed as Daily Growth

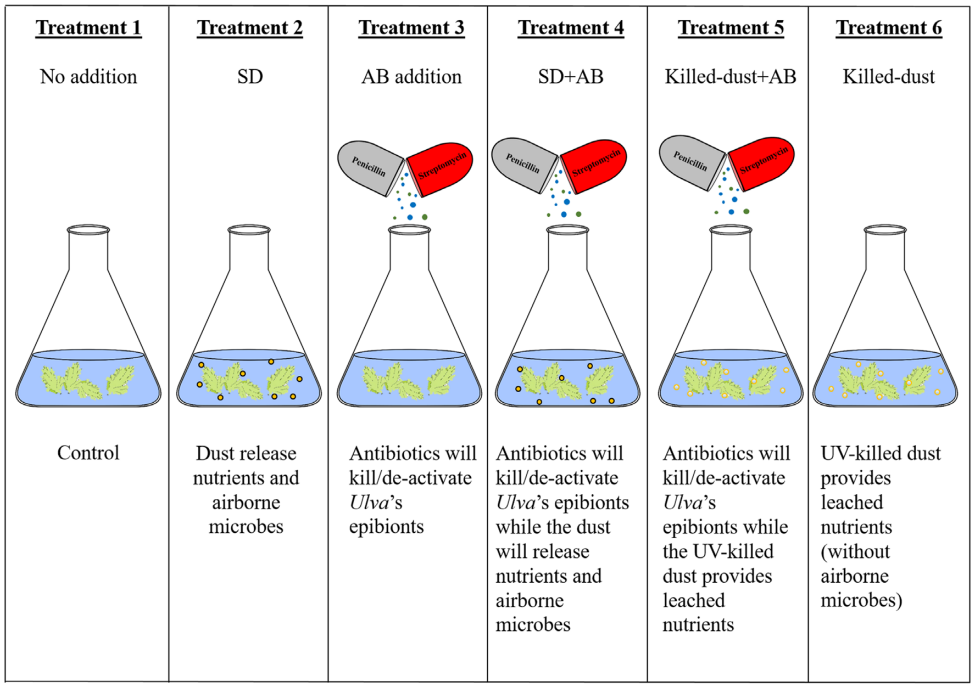

Figure 1. Experimental design include triplicates of: control (no additions, treatment 1), addition of Saharan Dust (SD) (treatment 2), pen-strep (AB) addition (treatment 3), addition of both AB and SD (treatment 4), addition of UV treated dust (killed-dust) and AB (treatment 5) and addition of killed dust only (treatment 6).

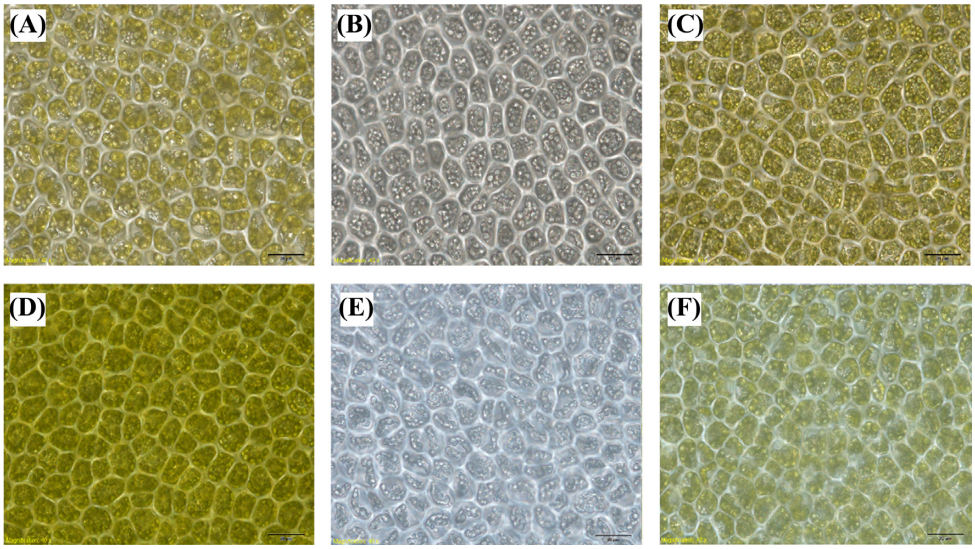

Figure 2. A tissue cross section of *U. Ohnoi* at the Control treatment **(A)**; AB addition **(B)**; SD addition **(C)**; SD+AB **(D)**; UV-treated SD+ AB **(E)**; and UV-treated SD **(F)** measured at the conclusion of the experiment (1 week) with a light microscope using a X20 magnification. Scale bar = 20 μm.

Rate (DGR) using the equation:

$$\mathrm{DGR} = \frac{\left(\frac{\mathrm{WT}-\mathrm{W0}}{\mathrm{W0}}\right)}{\mathrm{T}} \times 100$$

Where W_0 is the initial sample fresh-weight, W_T is the sample fresh-weight after the incubation (1 week) and T stands for the days between measurements.

Photosynthetic efficiency (Fv/Fm)

Photosynthetic responses were measured at the end of the incubation period (1-week post acclimation, similarly to the other variables) using Pulse Amplitude Modulated (PAM) fluorometry with a Diving PAM (Walz, Effeltrich, Germany) (Beer *et al.* 2000). Photosynthetic quantum yields (*Fv/Fm*) were measured after 30 min of dark acclimation and calculated as follows:

$$\mathrm{Yield\ (Fv/Fm)} = \frac{(\mathrm{Fm} - \mathrm{F0})}{\mathrm{Fm}}$$

Where Fm is the maximum fluorescence emission and F0 is the fluorescence before saturating light pulse was imposed ($F_m - F_0 = F_v$).

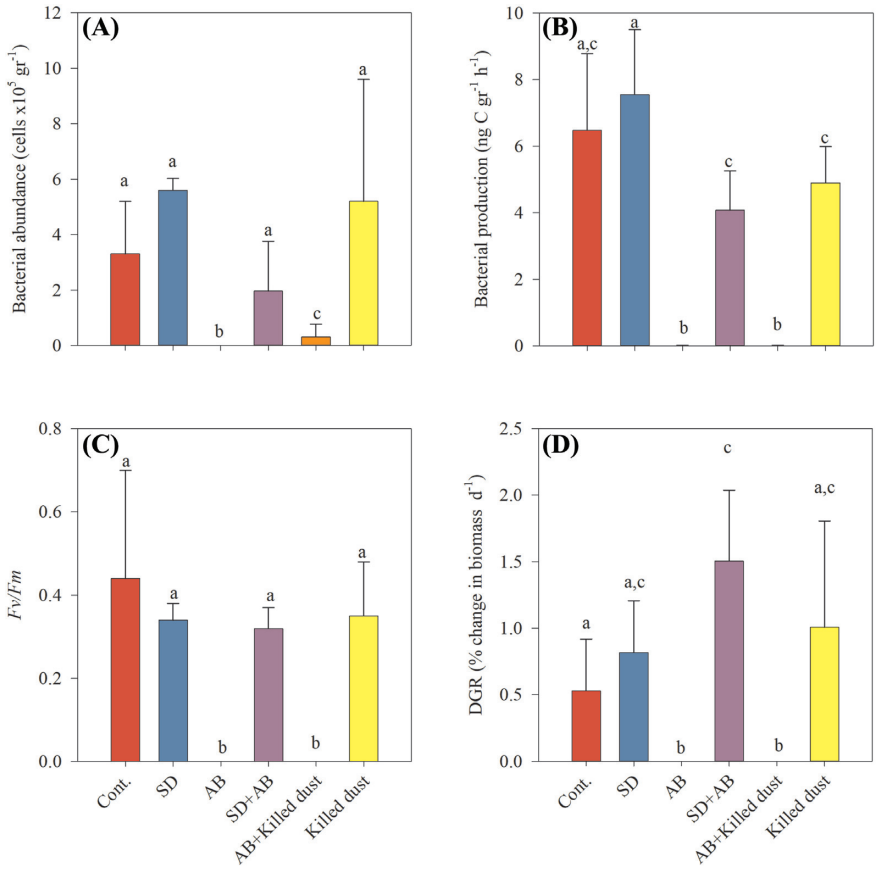

Figure 3. *Ulva ohnoi* microbial epibionts abundance **(A)**, epibionts bacterial production **(B)**, *Ulva*'s photosynthetic efficiency (*Fv/Fm*) **(C)** and specific growth rate **(D)** following the addition of dust (blue); AB (white); AB+dust (purple); AB+UV-treated dust (orange); UV-treated dust (yellow) and unamended control (red). Results presented are the averages and standard deviation of 3 independent triplicates measured at the conclusion of the experiment (1 week). The letters above the columns represent significant differences (one-way ANOVA and a Fisher LSD means comparison test, $P < 0.05$) for mean values between additions.

Epibionts microbial abundance

Ulva ohnoi associated bacterial cells were analyzed after a 1-week of incubation with the different treatments (Cont. SD, AB, etc.). To this end, *U. ohnoi* specimens (9 cm^2) collected from the different Erlenmeyer flasks were placed in 5 ml Eppendorf tubes containing 2 ml of FSW and sonicated for 5 min in a bath sonication system (Symphony, VWR). Preliminary experiments show that 5 min is the minimal time to sufficiently remove enough epibiotic bacteria from the algal surface for microbial abundance analysis yet not damage their cell wall that may result in underestimation of the actual abundance (~4-fold higher abundance compared to no sonication, Fig. S3, Supporting Information). We note that this sonication procedure may not necessarily remove all of the microbial cells attached to the *Ulva*'s surface, resulting in a possible underestimation of the total bacterial epibiont abundance (i.e. some bacteria may remain attached to the tissue). After sonication, 1.7 ml of the sample were transferred into new sterile cryo-vials, fixed with 6 μl of 50% glutaraldehyde (Sigma, G7651) and kept at 4°C until analysis within ~2–3 days. Before analysis, the samples were stained with the nucleic-acid SYBR green stain (Applied Biosystems cat #S32717) for 10 min in the dark. Stained samples were analyzed using an Attune Acoustic Focusing flow-cytometer (Applied Biosystems) by discrimination based on green fluorescence (530 nm) and side scatter (Hazan *et al.* 2018; Rahav *et al.* 2019b).

Bacterial production

Ulva ohnoi associated bacterial productivity was measured post-1-week of incubation for each treatment. Where, specimens from the different Erlenmeyer beakers (Cont. SD, AB, etc.) were suspended in 3.5 ml of FSW and spiked with 100 nmol L^{-1} (final concentration) of [4,5–^{3}H]-leucine (Perkin Elmer, specific activity 168 Ci mmol^{-1}). The samples were incubated in the dark for 4 h at room temperature. Leucine assimilation was halted by the addition of 100 µl of cold 100% trichloroacetic acid (TCA) solution (Sigma T0699). The *U. ohnoi* specimens were then sonicated (Symphony, VWR) for 10 minutes to remove the bacterial biomass from the outer macroalgae surface area. The biomass extract was divided into three 1 ml aliquots, and the micro-centrifugation technique was applied (Smith *et al.* 1992). Disintegration per minute (DPM) of the [4,5–^{3}H]-leucine from each sample was read using a liquid scintillation counter (Packard Tri carb 2100). A conversion factor of 1.5 kg C mol^{-1} with an isotope dilution factor of 2.0 was used to calculate bacterial carbon assimilation rate (Simon, Alldredge and Azam 1990).

Diversity of epibionts

DNA was extracted from triplicate samples of 1 cm^2 *U. ohnoi* thalli, using the PowerSoil^{TM} DNA Isolation Kit (QIAGEN, California, USA). We note that DNA extractions from the Saharan

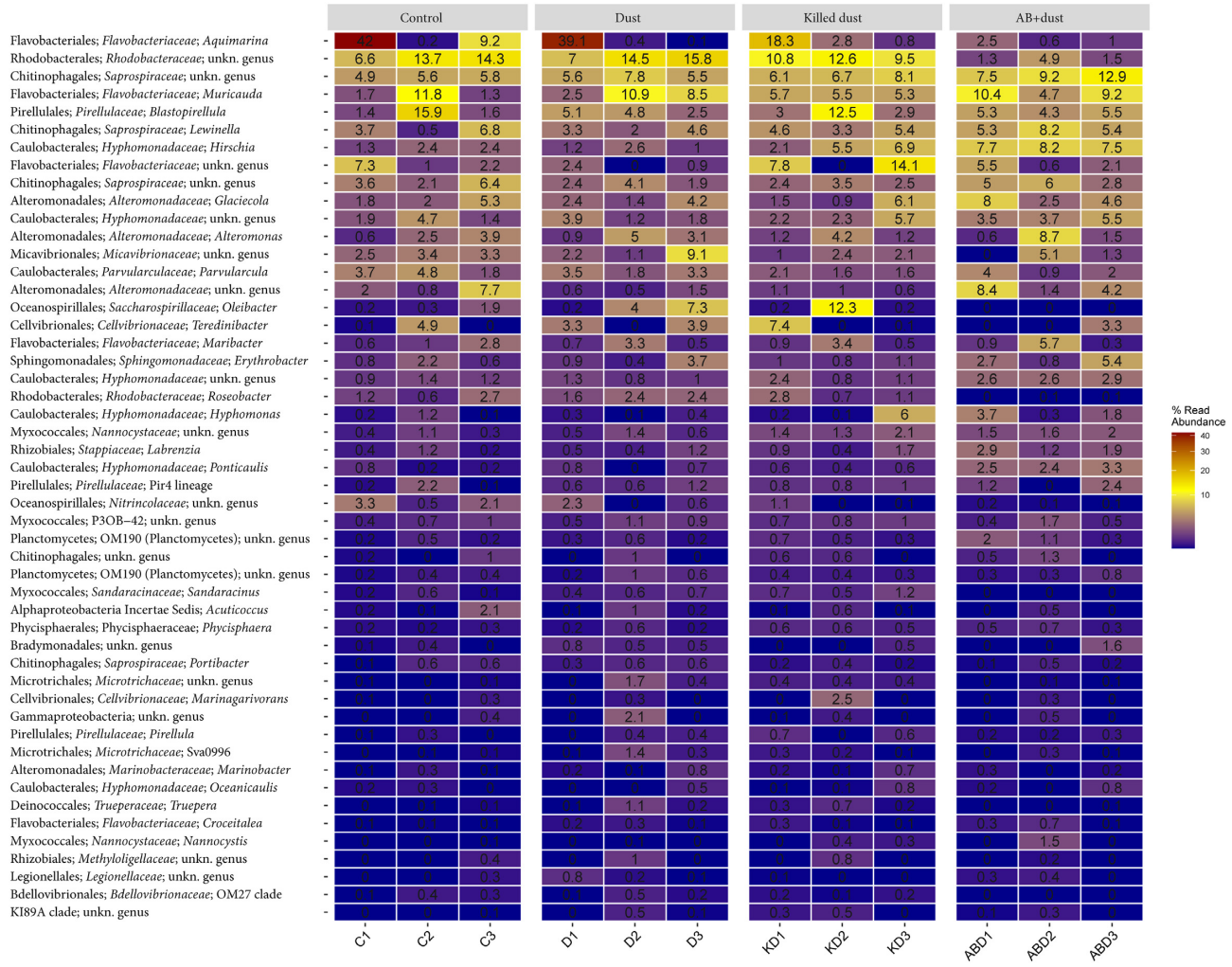

Figure 4. Relative ASVs abundance derived from 16S rRNA analyses from the different *U. ohnoi* treatments. Note that no sequences were amplified from the AB or AB+UV-treated dust treatments.

soils did not yield enough material for sequencing and therefore was not analyzed. DNA yields for treatments 'AB' and 'AB+UV-treated dust' were very low or unmeasurable (Fig. S4, Supporting Information). The V4 region of the 16S rRNA gene was amplified using the modified primer pair 515F-806R in combination with consensus sequences CS1 and CS2 (5′- ACACTGACGACATGGTT CTACAGTGYCAGCMGCCGCGGTAA, 5′- TACGGTAGCAGAGACTT GGTCTGGACTACNVGGGTWTCTAAT, Apprill *et al.* 2015; Parada, Needham and Fuhrman 2016), using the following PCR amplification protocol: initial denaturation at 94°C for 45 s, 30 cycles of denaturation (94°C for 15 s), annealing (15 cycles at 50°C and 15 cycles at 60°C for 20 s) and extension (72°C for 30 s). Library preparation and sequencing of 2 × 250 bp Illumina MiSeq reads were performed at HyLabs (Israel).

Between 19 000 and 59 000 raw reads were obtained. Reads were truncated based on quality plots, checked for chimeras, merged and grouped into amplicon sequence variants (ASVs) with Deblur (Amir *et al.* 2017), as implemented in QIIME2 V2019.7 (Rideout *et al.* 2018). Taxonomy was assigned to the representative sequences with a Naïve-Bayes classifier that was trained on the Silva 132 99% Operational Taxonomic Units from 515F/806R region of the 16S rRNA sequences. Downstream statistical analyses and plotting were performed in R (R Core Team 2020), using libraries phyloseq (McMurdie and Holmes 2013), ampvis2 (Andersen *et al.* 2018) and ggplot2 (Wickham 2009). Chloroplast sequences were omitted during downstream bioinformatics analyses, resulting in 1500–5400 final non-chloroplast reads per sample (Table S1, Supporting Information). Permutation multivariate analysis of variance (PERMANOVA) was based on Bray–Curtis dissimilarities. Systematic changes across experimental conditions were estimated with DESeq2 (Love, Huber and Anders 2014). Amplicon reads are available in NCBI Sequence Read Archive, BioProject PRJNA687378.

Statistical analyses

Bacterial abundance, production, photosynthetic efficiency and daily growth rate measurements were compared among the different treatments using analysis of variance (ANOVA) followed by a Student-Newman-Keuls (SNK) posthoc test ($P < 0.05$). Before analyses, the data was log-transformed to ensure normality distribution of residuals (Shapiro-Wilk test) and checked for homogeneity of variance. All analyses were done using the Microsoft XLSTAT software.

RESULTS AND DISCUSSION

Macroalgae such as *Ulva* sp. host dense epiphytic bacterial communities, reaching up to ~1 × 10^7 bacterial cells per cm^2 thalli (Armstrong, Rogerson and Leftley 2000; Bengtsson, Sjøtun and øvreås 2010). These epiphytic microbes play important roles in *Ulva's* physiological state and function. For example, epiphytic bacteria can promote *Ulva lactuca*'s growth and prevent the colonization of pathogens on its thalli (Lemos, Toranzo and Barja 1985). In the absence of specific epibionts, *Ulva mutabilis and Ulva intestinalis* germ cells develop into 'atypical' colonies with abnormal cell-walls (Ghaderiardakani, Coates and Wichard 2017). Also, quorum-sensing molecules synthesized by the epiphytic Actinobacteria affect the morphogenesis of *Ulva linza* (Marshall *et al.* 2006). In turn, *Ulva* provides epiphytic bacteria with nutrients, mostly as dissolved organic substrates, and thalli to live on (Lemos, Toranzo and Barja 1985; Ghaderiardakani, Coates and Wichard 2017). In agreement with these studies, we showed that once the *U. ohnoi*'s epibionts were deactivated or removed using antibiotics (Fig. S1, Supporting Information), its physiological state was greatly deteriorated (Figs. 2 and 3). Specifically, upon the addition of antibiotics (treatment AB), the abundance of epibiotic prokaryotes was reduced by ~80% and their production reduced by ~90%, resulting in a completely bleached *U. ohnoi* tissue (Fig. 2) and below detection values of DGR and *Fv/Fm* (Fig. 3). This was in contrast to *U. ohnoi* specimens grown under control conditions (i.e. only FSW), where the thalli tissue was green (Fig. 2), the DGR was ~0.5% d^{-1} and the *Fv/Fm* reached ~0.45 (Fig. 3). The physiological characteristics found in the control treatments indicated the *Ulva* cells were 'healthy'(Ghaderiardakani, Coates and Wichard 2017; Krupnik *et al.* 2018), highlighting the tight symbiotic relations between *Ulva* and its epibionts, and their importance for the macroalgae growth.

Millions of tons of dust are regularly transported from the Sahara and other nearby deserts and deposited in the Mediterranean Sea (Herut *et al.* 2005; Lawrence and Neff 2009; Ganor *et al.* 2010), and thus may affect downwind ecosystems including macroalgae turfs. Our results show that upon the addition of Saharan dust (SD), both epibiont's abundance (~5.6 × 10^5 cells gr^{-1}) and production (~7.5 ng C gr^{-1} h^{-1}) slightly increased relative to the unamended control (although not significantly, Fig. 3). This could be due to leaching of nutrients from the dust and/or by the addition of airborne bacteria that settle on the *Ulva* outer surface. Following the SD addition, *Fv/Fm* (0.34) and DGR (~0.8% d^{-1}) also remained overall similar to the control treatment (P<>0.05i>, Fig. 3). Amendment of the UV-treated Saharan dust', which effectively released nutrients from the dust particles but not the airborne microbs, resulted in green *Ulva*'s thalli, and *Fv/Fm* (0.35) and DGR (1.0% d^{-1}) values similar to the unamended controls (Figs. 2 and 3). The addition of UV-treated dust to the AB-treated sample, which supplied only minerals but not airborne microbes, resulted in bleaching of *U. ohnoi* tissue (Fig. 2) and *Fv/Fm* and DGR below detection (Fig. 3), as in *U. ohnoi* to which antibiotics were added. In line with these findings, DNA yields in the AB+killed dust and in the antibiotics treatments were very low and no PCR product was detected when visualizing the gel (Fig. S4, Supporting Information). When untreated dust (with the airborne microbial community) was added to *U. ohnoi* amended with antibiotics ('AB+Dust'), *Ulva*'s thalli remained green (Fig. 2), and the *Fv/Fm* (0.32) and DGR (1.6% d^{-1}) were well above detection limit (Fig. 3). Further, DGR was significantly higher than that measured in the control, while *Fv/Fm* remained similar (Fig. 3). These results suggest the recovery of microbial epibionts following the addition of Saharan dust or that the dust-borne bacteria (~1.9 × 10^5 cells gr^{-1}) replaced the *Ulva's* epibiont community and maintained the *Ulva* physiological state, in the absence of its original microbial communities which were removed by the AB treatment.

Addition of SD following treatment with antibiotic (SD+AB) were not only able to restore the viability *U. ohnoi* and the activity of the associated microbiota, but also the microbial diversity. The dominant taxa, including *Flavobacteriaceae* such as *Aquimarina and Muricauda*, *Rhodobactereraceae* genera, *Saprosiraceae* (*Lewinwlla*), *Pirellulaceae* (*Blastopirellula*), *Alteromonaceae* (*Glaciecola*, *Alteromonas*) and *Hyphomonadaceae* (e.g. *Hirschia*), as well as others were found in all treatments to which SD was added (Fig. 4). Most of these taxa are known to be associated with algae, often algicidal and capable of degrading complex organic compounds including algal polysaccharides, but also as enhancers of algal growth (Chen, Sheu and Sheu 2012; Stabili *et al.* 2017; Lian *et al.* 2018; Koch *et al.* 2019; Ihua *et al.* 2020; Purohit, Chattopadhyay and Teli 2020). Note that the microbial community structure in the 'AB+dust' treatment was significantly different only when compared to the other three treatments together (PERMANOVA, p = 0.048, Fig. S5). This was due to the enrichment of very few ASVs, attributed to *Ponticaulis* (*Hyphomonadaceae*, Alphaproteobacteria), *Hirschia* (*Hyphomonadaceae*, Alphaproteobacteria), and unidentified *Alteromonadaceae* genus (Gammaproteobacteria). The abovementioned statistical difference was mainly attributed to the depletion of some ASVs in the 'AB+dust' treatment, in particular, those annotated as *Saprospiraceae*, *Flavobacteriaceae*, *Parvularculaceae*, *Micavibrionaceae*, *Rhodobacteraceae*, *Sandaracinaceae*, *Nitrincolaceae* and *Saccharospirillaceae* lineages. However, if considering the adjusted P values, this list is overall very limited (Table S2, Supporting Information).

The recovery of the *U. ohnoi* epiphytic microbial community may be partially due to the re-introduction of microbial lineages through the dust. Several of the abovementioned bacterial families were previously found in dust samples (Kellogg and Griffin 2006; Katra *et al.* 2014; Gat *et al.* 2017), suggesting atmospheric deposition of aerosols can also be a source of these bacteria in addition of being a source for external nutrients. *Alteromonas* has been found to respond positively to dust additions in a mesocosm experiment at the NW Mediterranean Sea (DUNE project, Laghdass *et al.* 2011). *Rhodobacteraceae* and *Oceanospirillaceae* have been found to respond positively to both anthropogenic and Saharan dust addition in Mediterranean coastal waters (Marín-Beltrán *et al.* 2019). Both of these studies explained the increase in *Alteromonas*, *Rhodobacteraceae* or *Oceanospirillaceae* by the addition of aerosol-derived chemicals (nutrients). We surmise, however, that additional 'biological' pathway which introduces airborne microbes and/or enables the recovery of epibiont microbial populations following dust deposition to seawater/freshwater may also occur under some circumstances. The fact that killed dust was not able to revive the microbial communities rules out other mechanisms of recovery, such as, the survival of the few bacteria during the antibiotics treatment and maintenance of algae viability based on the toxicity of dust to the potential microbial pathogens (Paytan *et al.* 2009). Considering that aerosols from different geographical sources and atmospheric trajectories carry unique communities of microbial populations (Katra *et al.* 2014; Gat *et al.* 2017; Mescioglu *et al.* 2019a), diverse dust storms may result in unique and distinct impact on the epiphytic bacteria of algae such as *Ulva*. Thus, different dust storms may have both positive and negative effects on *Ulva*'s fitness, by introducing either airborne

vital microbes or bacterial/viral/fungal pathogens. We stress that the diversity, abundance, activity and viability of dust-borne microbes should be extensively studied in future ventures.

Our results show that dust particles may serve as a potential source for diverse populations of *Ulva*'s microbial epibionts. This is consistent with reports showing that 1–25% of the bacterial communities carried with the dust or aerosol particles remains viable during transport (Womack, Bohannan and Green 2010; Polymenakou 2012). These airborne microbes can facilitate the rapid turnover of organic carbon and inorganic nutrients (Rahav *et al*. 2016b) and can attach to macroalgae, which potentially makes them excellent colonizers of *Ulva* and likely other macroalgae species.

The recovery of *U. ohnoi* and positive change in the physiological indicators following the introduction of the airborne bacterial taxa to antibiotics-treated *U. ohnoi* suggests that the airborne bacteria can re-occupy the lost functional niche and thus replace the original *Ulva* microbiome. The fact that many ASVs were similar between the controls and the AB+dust treatment suggests that specific lineages may be selected, as opposed to the 'lottery theory' which suggests that algae acquire epibionts from its surrounding based on their functionality alone rather than a taxonomic specificity. Understanding the functional role of these microbial populations and the biochemical mechanisms involved to acquire epibionts bacteria by *Ulva* sp. would be valuable to the management of this ecologically important algal species.

Lastly, *Ulva* species inhabit remarkable variable marine aquatic environments ranging from intertidal, shallow rocky shores to subtidal areas <100 m (Wolf *et al*. 2012). Biogeographical studies suggest that the *Ulva*'s distribution is strongly impacted by anthropogenic activities, or can be found in pristine habitats (Kozhenkova, Chernova and Shulkin 2006; Scherner *et al*. 2013). Many of these habitats are also strongly affected by nearby deserts, such as the Gobi the Sahara deserts. We, therefore, surmise that airborne bacteria can be an important source of microbial partners to macroalgae.

SUPPLEMENTARY DATA

Supplementary data are available at *FEMSEC* online.

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Conflicts of interest. None declared.

REFERENCES

Amir A, McDonald D, Navas-Molina JA *et al*. Deblur rapidly resolves single-nucleotide community sequence patterns. Gilbert JA (ed). *mSystems* 2017;**2**:e00191–16.

Andersen KS, Kirkegaard RH, Karst SM *et al*. ampvis2: an R package to analyse and visualise 16S rRNA amplicon data. *bioRxiv* 2018:299537.

Anwar MA, Choi S. Gram-negative marine bacteria: Structural features of lipopolysaccharides and their relevance for economically important diseases. *Mar Drugs* 2014;**12**:2485–514.

Apprill A, Mcnally S, Parsons R *et al*. Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. *Aquat Microb Ecol* 2015;**75**:129–37.

Armstrong E, Rogerson A, Leftley JW. The abundance of heterotrophic protists associated with intertidal seaweeds. *Estuar Coast Shelf Sci* 2000;**50**:415–24.

Astrahan P, Herut B, Paytan A *et al*. The impact of dry atmospheric deposition on the sea-surface microlayer in the SE mediterranean sea: An experimental approach. *Front Mar Sci* 2016;**3**:e00222.

Beer S, Poryan O, Larsson C *et al*. Photosynthetic rates of ulva (chlorophyta) measured by pulse amplitude modulated (pam) fluorometry. *Eur J Phycol* 2000;**35**:69–74.

Beleneva IA, Zhukova NV. Bacterial communities of some brown and red algae from Peter the Great Bay, the Sea of Japan. *Microbiology* 2006;**75**:348–57.

Bengtsson MM, Sjøtun K, øvreås L. Seasonal dynamics of bacterial biofilms on the kelp Laminaria hyperborea. *Aquat Microb Ecol* 2010;**60**:71–83.

Bulleri F, Benedetti-Cecchi L, Acunto S *et al*. The influence of canopy algae on vertical patterns of distribution of low-shore assemblages on rocky coasts in the northwest Mediterranean. *J Exp Mar Bio Ecol* 2002;**267**:89–106.

Burke C, Thomas T, Lewis M *et al*. Composition, uniqueness and variability of the epiphytic bacterial community of the green alga Ulva australis. *ISME J* 2011;**5**:590–600.

Chen WM, Sheu FS, Sheu SY. Aquimarina salinaria sp. nov., a novel algicidal bacterium isolated from a saltpan. *Arch Microbiol* 2012;**194**:103–12.

Chien C, Mackey KR, Dutkiewicz S *et al*. Effects of african dust deposition on phytoplankton in the Western Tropical Atlantic Ocean off Barbados. *Glob Biogeochem Cycles* 2016;**30**:716–34.

Croft MT, Warren MJ, Smith AG. Algae need their vitamins. *Eukaryot Cell* 2006;**5**:1175–83.

Das P, Mukherjee S, Sen R. Antimicrobial potential of a lipopeptide biosurfactant derived from a marine Bacillus circulans. *J Appl Microbiol* 2008;**104**:1675–84.

Duce RA, LaRoche J, Altieri K *et al*. Impacts of atmospheric anthropogenic nitrogen on the open ocean. *Science* 2008;**320**:893–7.

Egan S, Harder T, Burke C *et al*. The seaweed holobiont: Understanding seaweed-bacteria interactions. *FEMS Microbiol Rev* 2013;**37**:462–76.

Egan S, Thomas T, Kjelleberg S. Unlocking the diversity and biotechnological potential of marine surface associated microbial communities. *Curr Opin Microbiol* 2008;**11**:219–25.

Ganor E, Osetinsky I, Stupp A *et al*. Increasing trend of African dust, over 49 years, in the eastern Mediterranean. *J Geophys Res Atmos* 2010;**115**:1–7.

Gat D, Mazar Y, Cytryn E *et al*. Origin-dependent variations in the atmospheric microbiome community in Eastern Mediterranean dust storms. *Environ Sci Technol* 2017;**51**:6709–6718.

Ghaderiardakani F, Coates JC, Wichard T. Bacteria-induced morphogenesis of Ulva intestinalis and Ulva mutabilis (Chlorophyta): a contribution to the lottery theory. *FEMS Microbiol Ecol* 2017;**93**:fix094.

Griffin DW. Atmospheric movement of microorganisms in clouds of desert dust and implications for human health. *Clin Microbiol Rev* 2007;**20**:459–77.

Haywood MDE, Vance D., Loneragan N. Seagrass and algal beds as nursery habitats for tiger prawns (Penaeus semisulcatus and P. esculentus) in a tropical Australian estuary. *Mar Biol* 1995;**122**:213–23.

Hazan O, Silverman J, Sisma-Ventura G *et al.* Mesopelagic prokaryotes alter surface phytoplankton production during simulated deep mixing experiments in Eastern Mediterranean Sea waters. *Front Mar Sci* 2018;**5**, DOI: 10.3389/fmars.2018.00001.

Hempel M, Blume M, Blindow I *et al.* Epiphytic bacterial community composition on two common submerged macrophytes in brackish water and freshwater. *BMC Microbiol* 2008;**8**:1–10.

Herut B, Collier R, Krom MD. The role of dust in supplying nitrogen and phosphorus to the southeast Mediterranean. *Limnol Oceanogr* 2002;**47**:870–8.

Herut B, Zohary T, Krom MDD *et al.* Response of East Mediterranean surface water to Saharan dust: On-board microcosm experiment and field observations. *Deep Res Part II Top Stud Oceanogr* 2005;**52**:3024–40.

Ihua MW, FitzGerald JA, Guihéneuf F *et al.* Diversity of bacteria populations associated with different thallus regions of the brown alga Laminaria digitata. *PLoS One* 2020;**15**:e0242675.

Israel A, Golberg A, Neori A. The seaweed resources of Israel in the Eastern Mediterranean Sea. *Bot Mar* 2019;**63**:85–95.

Jickells TD, An ZS, Andersen KK *et al.* Global iron connections between desert dust, ocean biogeochemistry, and climate. *Science* 2005;**308**:67–71.

Joint I, Tait K, Wheeler G. Cross-kingdom signalling: Exploitation of bacterial quorum sensing molecules by the green seaweed Ulva. *Philos Trans R Soc B Biol Sci* 2007;**362**:1223–33.

Katra I, Arotsker L, Krasnov H *et al.* Richness and diversity in dust stormborne biomes at the southeast mediterranean. *Sci Rep* 2014;**4**:5265.

Kellogg Ca, Griffin DW. Aerobiology and the global transport of desert dust. *Trends Ecol Evol* 2006;**21**:638–44.

Keshtacher-Liebson E, Hadar Y, Chen Y. Oligotrophic bacteria enhance algal growth under iron-deficient conditions. *Appl Environ Microbiol* 1995;**61**:2439–41.

Koch H, Dürwald A, Schweder T *et al.* Biphasic cellular adaptations and ecological implications of Alteromonas macleodii degrading a mixture of algal polysaccharides. *ISME J* 2019;**13**:92–103.

Kozhenkova SI, Chernova EN, Shulkin VM. Microelement composition of the green alga Ulva fenestrata from Peter the Great Bay, Sea of Japan. *Russ J Mar Biol* 2006;**32**:289–96.

Krause-Jensen D, Duarte CM. Substantial role of macroalgae in marine carbon sequestration. *Nat Geosci* 2016;**9**:737–42.

Krupnik N, Rinkevich B, Paz G *et al.* Native, invasive and cryptogenic Ulva species from the Israeli Mediterranean Sea: risk and potential NIMROD. *Mediterr Mar Sci* 2018;**19**:132–46.

Lachnit T, Blümel M, Imhoff JF *et al.* Specific epibacterial communities on macroalgae: Phylogeny matters more than habitat. *Aquat Biol* 2009;**5**:181–6.

Lachnit T, Meske D, Wahl M *et al.* Epibacterial community patterns on marine macroalgae are host-specific but temporally variable. *Environ Microbiol* 2011;**13**:655–65.

Laghdass M, Blain S, Besseling M *et al.* Effects of Saharan dust on the microbial community during a large in situ mesocosm experiment in the NW Mediterranean Sea. *Aquat Microb Ecol* 2011;**62**:201–13.

Lawrence CR, Neff JC. The contemporary physical and chemical flux of aeolian dust: A synthesis of direct measurements of dust deposition. *Chem Geol* 2009;**267**:46–63.

Lemos ML, Toranzo AE, Barja JL. Antibiotic Activity of Epiphytic Bacteria Isolated from Intertidal Seaweeds. *Microb Ecol* 1985;**11**:149–63.

Lian J, Wijffels RH, Smidt H *et al.* The effect of the algal microbiome on industrial production of microalgae. *Microb Biotechnol* 2018;**11**:806–18.

Li J, Cao J, Zhu YG *et al.* Global Survey of Antibiotic Resistance Genes in Air. *Environ Sci Technol* 2018;**52**:10975–84.

Longford SR, Tujula NA, Crocetti GR *et al.* Comparisons of diversity of bacterial communities associated with three sessile marine eukaryotes. *Aquat Microb Ecol* 2007;**48**:217–29.

Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* 2014;**15**:550.

Mackey KRM, Roberts K, Lomas MW *et al.* Enhanced solubility and ecological impact of atmospheric phosphorus deposition upon extended seawater exposure. *Environ Sci Technol* 2012;**46**:10438–46.

Marshall K, Joint I, Callow ME *et al.* Effect of marine bacterial isolates on the growth and morphology of axenic plantlets of the green alga Ulva linza. *Microb Ecol* 2006;**52**:302–10.

Marín-Beltrán I, Logue JB, Andersson AF *et al.* Atmospheric deposition impact on bacterial community composition in the NW Mediterranean. *Front Microbiol* 2019;**10**:858.

Matsuo Y, Suzuki M, Kasai H *et al.* Isolation and phylogenetic characterization of bacteria capable of inducing differentiation in the green alga Monostroma oxyspermum. *Environ Microbiol* 2003;**5**:25–35.

McMurdie PJ, Holmes S. Phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLoS One* 2013;**8**:e0061217.

Mescioglu E, Rahav E, Belkin N *et al.* Aerosol microbiome over the Mediterranean Sea: Diversity and abundance. *Atmosphere (Basel)* 2019a;**10**:440.

Mescioglu E, Rahav E, Frada MJ *et al.* Dust-Associated Airborne Microbes Affect Primary and Bacterial Production Rates, and Eukaryotes Diversity, in the Northern Red Sea: A Mesocosm Approach. *Atmo* 2019b;**10**:358.

Nakanishi K, Nishijima M, Nishimura M *et al.* Bacteria that induce morphogenesis in Ulva pertusa (Chlorophyta) grown under axenic conditions. *J Phycol* 1996;**32**:479–82.

Parada AE, Needham DM, Fuhrman JA. Every base matters: Assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. *Environ Microbiol* 2016;**18**:1403–14.

Paytan A, Mackey KRM, Chen Y *et al.* Toxicity of atmospheric aerosols on marine phytoplankton. *Proc Natl Acad Sci USA* 2009;**106**:4601–5.

Peter H, Hörtnagl P, Reche I *et al.* Bacterial diversity and composition during rain events with and without Saharan dust influence reaching a high mountain lake in the Alps. *Environ Microbiol Rep* 2014;**6**:618–24.

Polymenakou PN. Atmosphere: A source of pathogenic or beneficial microbes? *Atmosphere (Basel)* 2012;**3**:87–102.

Provasoli L, Pintner IJ. Bacteria induced polymorphism in an axenic laboratory strain of Ulva lactuca (chlorophyceae). *J Phycol* 1980;**16**:196–201.

Purohit J, Chattopadhyay A, Teli B. Metagenomic Exploration of Plastic Degrading Microbes for Biotechnological Application. *Curr Genomics* 2020;**21**:253–70.

Rahav E, Belkin N, Paytan A *et al.* Bacterioplankton response to desert dust deposition in the coastal waters of the southeastern Mediterranean Sea ; A four year in-situ survey. *Atmos* 2018a;**9**:1–15.

Rahav E, Belkin N, Paytan A *et al.* The Relationship between Air-Mass Trajectories and the Abundance of Dust-Borne Prokaryotes at the SE. *Atmosphere (Basel)* 2019a;**10**:280.

Rahav E, Ovadia G, Paytan A *et al.* Contribution of airborne microbes to bacterial production and N2 fixation in seawater upon aerosol deposition. *Geophys Res Lett* 2016a;**43**:1–9.

Rahav E, Ovadia G, Paytan A *et al.* Contribution of airborne microbes to bacterial production and N2 fixation in seawater upon aerosol deposition. *Geophys Res Lett* 2016b;**43**:e066898.

Rahav E, Paytan A, Chien C Te *et al.* The impact of atmospheric dry deposition associated microbes on the southeastern mediterranean sea surface water following an intense dust storm. *Front Mar Sci* 2016c;**3**:1–11.

Rahav E, Paytan A, Mescioglu E *et al.* Airborne microbes contribute to N2 fixation in surface water of the Northern Red Sea. *Geophys Res Lett* 2018b;**45**:2–10.

Rahav E, Silverman J, Raveh O *et al.* The deep water of Eastern Mediterranean Sea is a hotspot for bacterial activity. *Deep Sea Res Part II Top Stud Oceanogr* 2019b;**164**:135–43.

R Core Team. *R: A Language and Environment for Statistical Computing*. 2020.

Reche I, Ortega-Retuerta E, Romera O *et al.* Effect of Saharan dust inputs on bacterial activity and community composition in Mediterranean lakes and reservoirs. *Limnol Oceanogr* 2009;**54**:869–79.

Rideout JR, Dillon MR, Bokulich NA *et al.* QIIME 2 : Reproducible, interactive, scalable, and extensible microbiome data science. *PeerJ Prepr* 2018, DOI: 10.7287/peerj.preprints.27295.

Scherner F, Ventura R, Barufi JB *et al.* Salinity critical threshold values for photosynthesis of two cosmopolitan seaweed species: Providing baselines for potential shifts on seaweed assemblages. *Mar Environ Res* 2013;**91**:14–25.

Schiel DR, Foster MS. The population biology of large brown seaweeds: Ecological consequences of multiphase life histories in dynamic coastal environments. *Annu Rev Ecol Evol Syst* 2006;**37**:343–72.

Simon M, Alldredge AL, Azam F. Bacterial carbon dynamics on marine snow. *Mar Ecol Prog Ser* 1990;**65**:205–11.

Smith DC, Smith DC, Azam F *et al.* A simple, economical method for measuring bacterial protein synthesis rates in seawater using 3H-leucine. *Mar Microb food web* 1992;**6**:107–14.

Stabili L, Rizzo L, Pizzolante G *et al.* Spatial distribution of the culturable bacterial community associated with the invasive alga Caulerpa cylindracea in the Mediterranean Sea. *Mar Environ Res* 2017;**125**:90–8.

Staufenberger T, Thiel V, Wiese J *et al.* Phylogenetic analysis of bacteria associated with Laminaria saccharina. *FEMS Microbiol Ecol* 2008;**64**:65–77.

Tujula NA, Crocetti GR, Burke C *et al.* Variability and abundance of the epiphytic bacterial community associated with a green marine Ulvacean alga. *ISME J* 2010;**4**:301–11.

Wahl M, Goecke F, Labes A *et al.* The second skin: ecological role of epibiotic biofilms on marine organisms. *Front Microbiol* 2012;**3**:292.

Weinberger F, Beltran J, Correa JA *et al.* Spore release in Acrochaetium sp. (Rhodophyta) is bacterially controlled. *J Phycol* 2007;**43**:235–41.

Wickham H. *Ggplot2: Elegant Graphics for Data Analysis*. New York: Springer-Verlag, 2009.

Wolf MA, Sciuto K, Andreoli C *et al.* Ulva (Chlorophyta, Ulvales) Biodiversity in the North Adriatic Sea (Mediterranean, Italy): Cryptic Species and New Introductions. *J Phycol* 2012;**48**:1510–21.

Womack AM, Bohannan BJM, Green JL. Biodiversity and biogeography of the atmosphere. *Philos Trans R Soc Lond B Biol Sci* 2010;**365**:3645–53.