



# The Impact of Dry Atmospheric Deposition on the Sea-Surface Microlayer in the SE Mediterranean Sea: An Experimental Approach

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The oligotrophic southeastern Mediterranean Sea (SEMS) is frequently exposed to desert-dust deposition which supplies nutrients, trace metals and a wide array of viable airborne microorganisms. In this study, we experimentally examined the impact of aerosol addition, collected during an intense dust storm event in early September 2015, on the biomass and activity of pico-phytoplankton and heterotrophic bacterial populations at the sea-surface micro layer (SML) relative to the sub surface layer (SSL). Aerosol (1.5 mg L<sup>-1</sup>) was added to SML and SSL water samples in microcosms (4.5 L) and the water was frequently sampled over a period of 48 h. While the aerosol amendment triggered a moderate 1.5–2-fold increase in primary production in both the SML and the SSL, bacterial production increased by ~3 and ~7-folds in the SSL and SML, respectively. Concurrently, the abundance and flow-cytometric characteristics (green fluorescence and side scatter signals) of high nucleic acid (HNA) and low nucleic acid (LNA) bacterial cells showed a significant increase in the %HNA, in both SML and SSL samples following aerosol amendment. This shift in nucleic acid content took place at a much faster rate in the SML, suggesting a more active heterotrophic community. These changes were likely a result of higher rates of carbon utilizations in the SML following the dust addition, as assessed by a selected hydrocarbons and saccharides analysis. Additionally, a high absorption rate of hydrocarbons by the aerosol particles was measured following the additions, leaving less than 10% of these molecules available for potential heterotrophic microbial utilization. Our results suggest that the heterotrophic microbial community inhabiting the SML is more efficient in utilizing aerosol associated constituents than the community in the SSL.

**Keywords:** microlayer, aerosols, microcosms, mediterranean region, bacterial productivity

## INTRODUCTION

The sea-surface microlayer (SML) is the uppermost layer of the oceans (20–400 μm thick), located between the subsurface layer (SSL) waters and the atmosphere (Liss and Duce, 1997). The SML is a unique physiochemical and biological habitat that covers ~70% of Earth's surface. This boundary layer may play a significant role in many biogeochemical processes including air-sea gas and heat

exchange (Liss and Duce, 1997) and the cycling of various elements (Wurl and Holmes, 2008). Its chemical content is different from the one usually found in the SSL. For example, higher concentrations of saccharides, hydrocarbons, amino acids, and polysaccharides were detected at the SML compared to the SSL (Engel et al., 2004). Hydrocarbons in the form of alkyl chains (i.e., n-alkanes) are highly enriched in the microlayer due to their hydrophobic character and low density (Marty and Saliot, 1976). It is assumed that high concentrations of organic matter in the SML may aggregate with sticky Transparent Exopolymer Particles (TEP) that are also enriched in the SML, resulting in gel-like particulate matter (Cunliffe et al., 2010). These aggregates may also include bacteria cells (Passow and Alldredge, 1994) and potentially various other molecules. In addition bacteria cells embedded in these particles may use these hydrocarbon molecules in the aggregates as an energy source (Grossi et al., 2007; Yakimov et al., 2007; Sevilla et al., 2015). Unsaturated alkyl chain like molecules as in the case of  $\alpha$ -olefins (1-alkenes), might show elevated photo-oxidation rates and as a result light induced degradation (Mouzdahir et al., 2001). These differences may affect microbial activity (Reinthalder et al., 2008; Sarmiento et al., 2015), enhance/reduce extracellular enzymatic activity (Kuznetsova and Lee, 2001; Engel and Galgani, 2016) and affect microbial diversity and abundance (Reinthalder et al., 2008; Vilacosta et al., 2013).

The SML communities (frequently termed “Neuston”) are composed of diverse groups of phytoneston (autotrophs) and bacterioneuston (heterotrophs) (Liss and Duce, 1997; Cunliffe et al., 2010), which thrive on the relatively enriched organic matrix (Guitart et al., 2013; Engel and Galgani, 2016). One of the main external sources of nutrients to the SML is wet or dry atmospheric depositions (Cunliffe et al., 2010; Guieu et al., 2014). Deposited aerosols first interact with the SML and then sink through the SSL to deeper waters, supplying nutrients such as N, P, and Fe (e.g., Herut et al., 1999, 2002; Chien et al., 2016). They may also introduce viable airborne microbes (Griffin, 2010; Peter et al., 2014; Rahav et al., 2016a,b). Therefore, any dry or wet deposition has the potential of changing the diversity and activity of the phytoneston and bacterioneuston communities.

The southeastern Mediterranean Sea (SEMS) is a low nutrients low chlorophyll marine province (Berman et al., 1984; Krom et al., 1991; Yacobi et al., 1995; Rahav et al., 2013; Kress et al., 2014) dominated by small-size microbes with low productivity (Yacobi et al., 1995; Bar-zeev and Rahav, 2015; Raveh et al., 2015). Recent studies showed that these autotrophic microorganisms are primarily limited by N or co-limited by N&P, whereas heterotrophic bacteria are P or C limited during summertime (Kress et al., 2005; Zohary et al., 2005; Rahav et al., 2016c). Previous studies emphasized the significant role of atmospheric deposition in supplying limiting nutrients to the SEMS (Herut et al., 1999, 2002; Guieu et al., 2014). While these studies have assessed the impact of atmospheric dust deposition on surface phytoplankton and heterotrophic bacterial communities in the SEMS (e.g., Ridame et al., 2011; Rahav et al., 2016b), none of these studies distinguished between the SML and the SSL.

In this study, we present a microcosm experimental assay that examines the response of the neuston and bulk bacterioplankton communities, collected from the SML and SSL respectively

to the addition of dry deposition of aerosol including desert dust ( $1.5 \text{ mg L}^{-1}$ ). Temporal dynamics of the autotrophic and heterotrophic microbial abundances and activity were recorded for 48 h at high temporal resolution (every 4–8 h). High nucleic acid (HNA) concentration per bacterial cell, was reported as an efficient measure for bacterial activity (Lebaron et al., 2001; Talarmin et al., 2011; Van Wambeke et al., 2011) and thus was included in this study in addition to bacterial productivity. Chemical analyses of saccharides and selected hydrocarbons were also examined. We hypothesized that due to the physiochemical and biological differences between the SML and SSL, any external atmospheric addition that delivers micro and macro-nutrients may trigger distinct responses in these two layers.

## MATERIALS AND METHODS

### SML and SSL Sampling

SML water samples were collected on December 15, 2015 by using a custom made rotating drum sampler as described in Harvey (1966) with minor changes: the rotating drum used was a glass tube ( $r = 18 \text{ cm}$ ;  $l = 60 \text{ cm}$ ). The drum was pre-cleaned with a concentrated HCl solution and washed with sample water for a few min prior collection. No silicon or plastic tubes were used. The collector includes an indurated non-contaminating silicon blade (0.5 m) fixed on an aluminum grip holder, collecting the water into a pre-combusted glass bottle. These changes are consistent with the glass plate collection procedure (Harvey and Burzell, 1972) by using similar materials. In addition, these improvements allow for a fast cleaning procedure, avoiding the contamination caused by reusing materials such as plastics and silicon tubing. The SML sample (23 L in total) was collected by connecting the sampler in parallel to a small boat (rotating speed  $\sim 5 \text{ RPM}$ ). SML thickness was approximately  $75 \mu\text{m}$  thick, and collection time was 90 min. Sampling starting point selected was 1 km offshore ( $32^\circ 49' 34\text{N}$ ,  $34^\circ 57' 20\text{E}$ ); collection ended 560 m northeast of the starting point. The study area is a coastal oligotrophic water zone (see discussion below); with low influence of urban runoff. The port of Haifa is located a few kilometers north of our study area; however, the general water circulation carries most of the bulk water away from our study zone. The average bottom depth along the sampling was 13 m. SSL water sample (23 L in total) was collected from 1 m depth along the same cruise-track. Samples were kept in acid prewashed sealed containers until processed at the Israel Oceanographic and Limnological Research (IOLR) institute. The wind speed was  $< 3.5$  knots, with waves of up to 0.3 m, and the seawater surface temperature was  $18^\circ\text{C}$ . Though waves in the same magnitude showed no impact on SML surfactants tension and spreading rates (Hale and Mitchell, 1997), it is possible that the SML collection may include minor amounts of SSL water and thus the results presented here may be an underestimation of the trends observed (i.e., dilution of the microlayer water with sub-surface water).

### Aerosol Collection and Experimental Design

A dry deposition sample was collected on September 8–9, 2015, using a pre-clean  $2 \text{ m}^2$  glass plate (more details in Rahav

et al., 2016b). The pre cleaned glass plate was exposed for 48 h during an extreme dust storm arriving from the north-east (**Supplementary Figure S1**). Over this time  $1 \text{ gr m}^{-2}$  of aerosol was deposited of plate area. Assuming a deposition rate of up to  $50 \text{ g dust m}^{-2} \text{ yr}^{-1}$  in the Mediterranean Sea (Lawrence and Neff, 2009), the accumulated aerosol in this time period corresponds to  $\sim 2\text{--}3\%$  of the annual dust deposition in this system. We added  $1.5 \text{ mg L}^{-1}$  of the collected aerosol (in triplicate) to acid-cleaned 4.5 L transparent High-density polyethylene Nalgene bottles containing either SML or SSL water ( $6.7\text{--}6.8 \text{ mg}$  aerosol in total to each bottle). This addition is equivalent to the concentration reported for the upper mixed layer (top 5 m) during a heavy dust storm (Herut et al., 2002, 2005; Rahav et al., 2016b), and is similar to the amounts tested in other studies from the SEMs (Ridame et al., 2011; Herut et al., 2016). Samples were incubated in an outdoor pool with seawater flow-through to maintain ambient seawater temperature. The bottles used allowed the penetration of most of the light spectrum, excluding UV light. Blank treatments of SML or SSL water without an aerosol addition were also carried out in parallel. SSL bottles were submerged to a depth of 1 m in the pool by using weights. Subsamples of seawater from each incubation bottle were collected for *Synechococcus* abundance, pico-eukaryotes abundance, nano-eukaryotic abundance, heterotrophic bacterial abundance, primary production, and bacterial production measurements at 0, 1.5, 5, 9, 17, 21, 26, and 44 h after the aerosol addition.

### Pico-Phytoplankton and Bacterial Abundance

Water samples (1.8 mL) were fixed with 50% glutaraldehyde ( $6 \mu\text{L}$ , Sigma-Aldrich G7651), frozen in liquid nitrogen and stored at  $-80^\circ\text{C}$  until analyzed. Pico-phytoplankton abundance (namely *Synechococcus* and autotrophic eukaryotes algae) was determined using an Attune<sup>®</sup> Acoustic Focusing Flow Cytometer (Applied Biosystems) equipped with a syringe-based fluidic system and 488 and 405-nm lasers at a flow rate of  $100 \mu\text{L min}^{-1}$  (Bar-zeev and Rahav, 2015). For heterotrophic bacterial abundance determination, subsamples ( $100 \mu\text{L}$ ) were separately incubated at room temperature for 15 min with the nucleic acid stain SYTO9 ( $1:10^5$  vol:vol) and then run at a low flow rate of  $25 \mu\text{L min}^{-1}$  (Vaulot and Marie, 1999). Low nucleic acid (LNA) and HNA bacteria were differentiated by coupling their green fluorescence and side-scatter (Lebaron et al., 2001; Talarmin et al., 2011; Van Wambeke et al., 2011). For more details, see (Rahav et al., 2016b).

### Primary Production

Photosynthetic carbon fixation rates were estimated using the  $^{14}\text{C}$  incorporation method (Nielsen, 1952). For more details, see (Rahav et al., 2016b).

### Bacterial Production

Bacterial production was estimated using the  $[4,5\text{-}^3\text{H}]$ -leucine incorporation method (Simon et al., 1990). A conversion factor of  $3 \text{ g C mol}^{-1}$  leucine incorporated was used, assuming an

isotopic dilution of 2.0 (Simon and Azam, 1989). For more details, see (Rahav et al., 2016b).

### Dissolved Monosaccharides Concentration

SML and SSL samples (10 ml) were filtered using  $0.22 \mu\text{m}$  polycarbonate membranes (Osmonics INC). The dissolved monosaccharides' (no hydrolysis) concentration was determined using the 2,4,6-Tripyridyl-s-Triazine (TPTZ) reagent (Sigma-Aldrich) method according to Mykkestad et al. (1997). All samples were analyzed using an Uvikon 9100/9400 spectrophotometer (SECOMAM).

### GC/MS Analysis

Samples (200 ml) were filtered using  $0.22 \mu\text{m}$  non-absorbing membrane, followed by the extraction of dissolved n-alkanes and  $\alpha$ -olefins from the water samples by liquid:liquid extraction, using  $3 \times 30 \text{ ml}$  n-hexane extraction repeats, followed by  $3 \times 30 \text{ ml}$  dichloromethane. The organic extracts were combined, concentrated to 1 ml via rotary evaporation and dried by passing through an  $\text{MgSO}_4$  column. Samples were analyzed using an Agilent 6890 gas chromatograph coupled with a 5973 Agilent mass spectrometer. Identification of the hydrocarbons was based on ions analysis (alkane ions = 43, 57, 71...  $\alpha$ -olefins = 41, 55, 69...) in addition to library matching (NIST 2) and external standards R.T as described next. Quantification of the results was achieved by using  $\text{C}_8\text{--}\text{C}_{30}$  external standards of n-alkanes (Sigma-Aldrich) and  $\alpha$ -olefins (AccuStandard<sup>®</sup>, Inc). Alkanes and  $\alpha$ -olefins adsorption to the membrane and to the experiment flasks was lower than 10%, method quantitation level =  $10 \text{ ng L}^{-1}$ .

### Statistical Analyses

The different variables presented in the figures and tables are averages and standard deviation (biological replicates,  $n = 3$ ). Changes in primary production, bacterial production, the abundance of *Synechococcus*, pico/nano-eukaryotes, and heterotrophic bacteria throughout the experiments (0–44 h) were evaluated using a one-way analysis of variance (ANOVA), followed by a Fisher LSD multiple comparison *post hoc*-test with a confidence level of 95% ( $\alpha = 0.05$ ). Prior analyses, the ANOVA assumptions were examined. The statistical analyses were carried out using the XLSTAT software.

## RESULTS

### Initial SML and SSL Characteristics Prior to Aerosol Addition

The SML at the study site was characterized by a higher concentration of n-alkanes ( $52.5 \text{ ng L}^{-1}$ ) and olefins, also termed "1-alkenes" ( $1.12 \text{ mg L}^{-1}$ ), relative to the SSL ( $46.4 \text{ ng n-alkanes L}^{-1}$  and  $0.70 \text{ mg } \alpha\text{-olefins L}^{-1}$ ). Similarly, the SML contained a higher concentration of monosaccharides relative to the SSL,  $890 \mu\text{g L}^{-1}$  vs.  $613 \mu\text{g L}^{-1}$ , respectively (**Table 1**). The total  $\alpha$ -olefins (1-alkenes) concentration in the SML was more than 3 orders of magnitude higher than the alkanes and monosaccharides, suggesting  $\alpha$ -olefins were a dominant source of carbon (**Table 1**).

**TABLE 1 | Dissolved  $\Sigma$  [alkanes<sub>(C<sub>8</sub>-C<sub>30</sub>)</sub>],  $\Sigma$  [ $\alpha$ -olefins<sub>(C<sub>11</sub>-C<sub>32</sub>)</sub>] and monosaccharides in the SML and SSL, prior (T0) and 44 h after (T44) aerosol addition.**

Variable	Unit	Prior aerosol addition (0 h)			End of incubations (44 h)		
		SML	SSL	EF	SML	SSL	EF
Total n-alkanes (C <sub>8</sub> -C <sub>30</sub> )	ng L <sup>-1</sup>	52.5	46.4	1.13	23.7	n.d	>2.37
Total $\alpha$ olefins (C <sub>8</sub> -C <sub>30</sub> )	mg L <sup>-1</sup>	1.12	0.70	1.60	0.01	n.d	>1000
Monosaccharides	$\mu$ g L <sup>-1</sup>	890	613	1.45	472	190	2.48

EF, enrichment factor (SML: SSL ratio).

n.d, not detected- below the quantitation level of 10 ng L<sup>-1</sup>.

*Synechococcus* dominated the picophytoplankton in the SML and SSL ( $\sim 2.3 \times 10^4$  cells ml<sup>-1</sup>), whereas in both layers the pico-eukaryotes abundance was lower by an order of magnitude ( $1.0 \times 10^3$  to  $1.5 \times 10^3$  cells ml<sup>-1</sup>) (Table 2). Heterotrophic bacterial abundance was similar in both water layers ( $\sim 1.5 \times 10^5$  cells ml<sup>-1</sup>), although high nucleic acid bacteria (HNA) accounted for 7% at the SML and  $\sim 15\%$  at the SSL. Primary production (PP) was overall low, with 60% higher rates measured at the SML ( $0.08 \pm 0.00 \mu\text{g C L}^{-1} \text{ h}^{-1}$ ) compared to the SSL ( $0.05 \pm 0.00 \mu\text{g C L}^{-1} \text{ h}^{-1}$ ) (Table 2). Similarly, bacterial production (BP) was  $\sim 50\%$  higher at the SML ( $0.06 \pm 0.00 \mu\text{g C L}^{-1} \text{ h}^{-1}$ ) than at the SSL ( $0.04 \pm 0.01 \mu\text{g C L}^{-1} \text{ h}^{-1}$ ) (Table 2). Nevertheless, these activity rates resulted in a similar BP:PP ratio ( $\sim 0.75:1$ ), suggesting a weak dominance of autotrophic metabolism (over heterotrophic metabolism) in both water layers.

## Aerosol Characteristics

The aerosol sample used in our experiment was collected during an exceptional dust storm event that arrived from the northeast rather than the more common southwest (Saharan desert) sources (Supplementary Figure S1). Based on the aluminum (Al) concentration in the collected aerosol, the Al settling velocity, and the weight of the particles collected, we calculated a dry deposition concentration of 1.05 mg L<sup>-1</sup> at the upper 5 m mixed layer as reported in Rahav et al. (2016b). While such an episodic and strong deposition event is considered high in this system (reviewed in Guieu et al., 2014), it is well in the range previously reported for the Mediterranean Sea (e.g., Herut et al., 2002; Ridame et al., 2011, 2013) and similar to additions tested in previous microcosm bioassays (Herut et al., 2005; Rahav et al., 2016c). The micro and macro solubilized nutrients concentrations that were leached off the added aerosol sample are detailed in Rahav et al. (2016b) and discussed below.

## Bacterioplankton Response to Aerosol Addition

Following aerosol addition, the abundances of *Synechococcus* (ranging from  $\sim 2 \times 10^4$  cells ml<sup>-1</sup> to  $\sim 7 \times 10^4$  cells ml<sup>-1</sup>) and nano-eukaryote (ranging from 250 cells ml<sup>-1</sup> to 1200 cells ml<sup>-1</sup>) in both layer samples were similar to the unamended treatments (Figure 1) and thus they were not significantly affected by the addition of the aerosols. Pico-eukaryote abundance following the aerosol addition was similar to the unamended control during the first 21 h in both layers ( $\sim 1000$ – $2000$  cells ml<sup>-1</sup>,  $P > 0.05$ ), thereafter increased  $\sim 2$ -fold relative to the unamended controls in the SML microcosms only (5000 cells ml<sup>-1</sup>,  $P <$

0.01) (Figures 1B,E, Table 3). Heterotrophic bacterial abundance increased  $\sim 2$ -fold in the SML and SSL following the addition of aerosol (from  $\sim 1.5 \times 10^5$  cells ml<sup>-1</sup> to  $\sim 4 \times 10^5$  cells ml<sup>-1</sup>,  $P < 0.05$ ), with more profound differences between the treated versus the non-treated sample for the SML (Figures 2A,C). These differences were also apparent in the increased relative abundance of HNA over LNA bacteria, with 2-fold higher HNA bacteria ( $P = 0.02$ ) recorded following the aerosol addition at both the SML and the SSL (Figure 2, Table 3). While the changes in microorganism abundance were relatively modest (Figures 1, 2), more profound differences were recorded for both PP and BP rates following the aerosol addition (Figure 3, Table 3). PP rates increased by  $\sim 2$ -fold relative to the untreated control ( $P < 0.05$ ) 17 h post aerosol addition in both the SML and SSL. This enhancement lasted for the experiment's entire duration; 44 h post addition (Figures 3A,D, Table 3). Simultaneously, BP exhibited a stronger response to the aerosol addition,  $\sim 3$ -fold higher rates recorded at the SSL (increasing from  $0.04 \mu\text{g C L}^{-1} \text{ h}^{-1}$  to  $0.11 \mu\text{g C L}^{-1} \text{ h}^{-1}$ ,  $P < 0.05$ ) and up to a  $\sim 7$ -fold increase at the SML (from  $0.06 \mu\text{g C L}^{-1} \text{ h}^{-1}$  to  $0.44 \mu\text{g C L}^{-1} \text{ h}^{-1}$ ,  $P < 0.05$ ) relative to the unamended control that showed no change (Figures 3B,E, Table 3). These differences resulted in a higher BP:PP ratio at both water layers following the aerosol addition relative to the unamended controls (Figure 3,  $P < 0.05$ ). Enrichment factors (EF) were calculated as the ratio between the abundance or the activity in the SML divided by those measured in the SSL (0–44 h). The EF-values for *Synechococcus* and nano-eukaryotes were similar in both the unamended and the aerosol addition bioassays (an insignificant change from a 1:1 ratio,  $P > 0.05$ ). In contrast, pico-eukaryotes abundance, heterotrophic bacterial abundance, PP, and BP all exhibited significant higher EF-values ( $> 1$ ) following aerosol addition (Figure 4,  $P < 0.05$ ).

## Dissolved Hydrocarbons Post Aerosol Addition

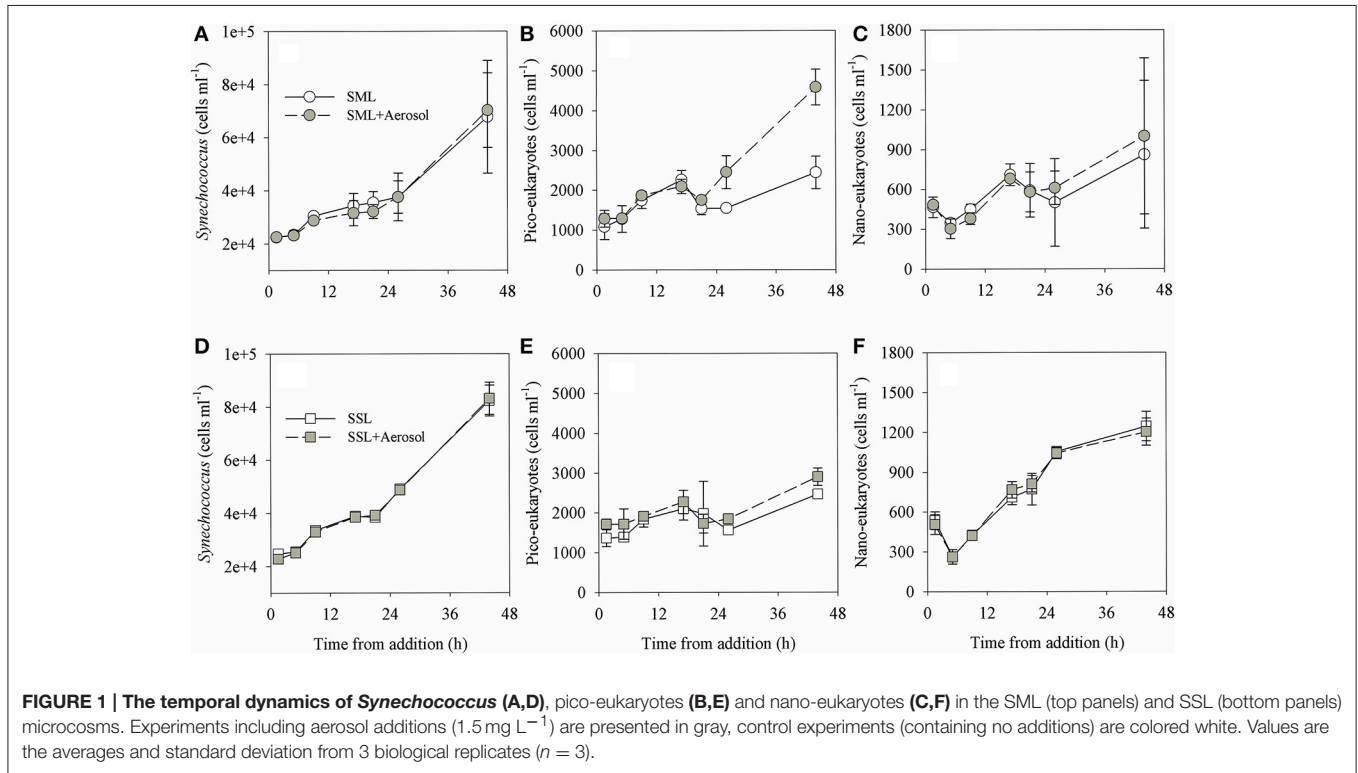
Overall, the dissolved n-alkane concentration decreased substantially with time (T0 vs. T44 h) in the SML and to a lower level in the SSL following aerosol additions (Table 1). n-alkanes at the SML were reduced by  $\sim 50\%$  at the conclusion of the experiment (reaching 23.7 ng L<sup>-1</sup>), whereas a decrease to below detection limit was observed in the SSL (Table 1).  $\alpha$ -olefins, which were the major carbohydrate found, were reduced by two orders of magnitude in the SML and to below detection limit in the SSL at T44 h (Table 1). Finally, monosaccharides decreased by  $\sim 50\%$  at the SML ( $472 \mu\text{g L}^{-1}$ ) and by  $\sim 66\%$  at the SSL ( $190 \mu\text{g L}^{-1}$ ) (Table 1). Based on the BP rates measured



**TABLE 2 | Initial biological characteristics of the SML and SSL prior aerosol addition (T0).**

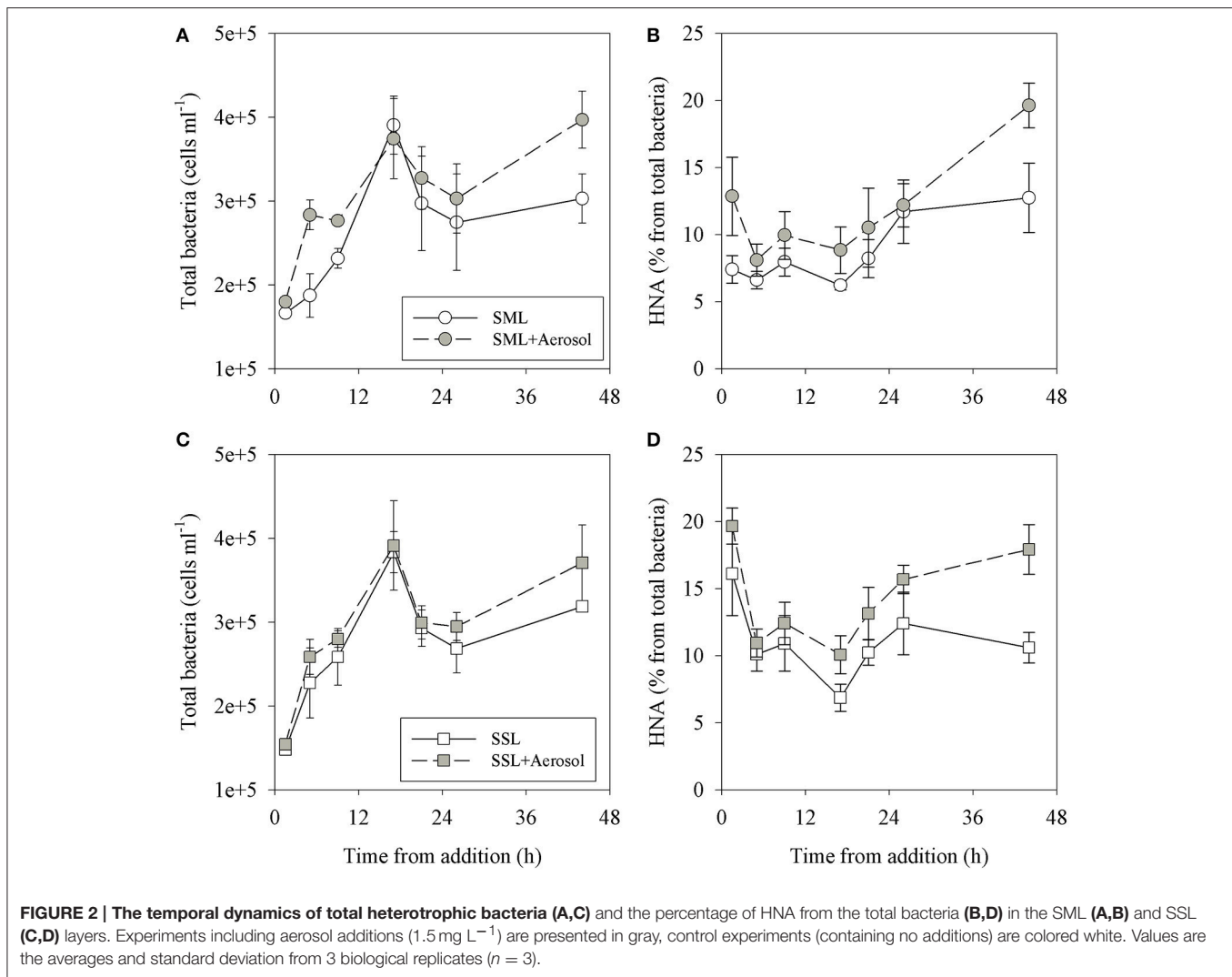
Water layer	BP ( $\mu\text{g C L}^{-1} \text{h}^{-1}$ )	PP ( $\mu\text{g C L}^{-1} \text{h}^{-1}$ )	Total bacteria (cells $\text{ml}^{-1}$ )	<i>Synechococcus</i> (cells $\text{ml}^{-1}$ )	pico-eukaryotes (cells $\text{ml}^{-1}$ )
SML	0.06 $\pm$ 0.00	0.08 $\pm$ 0.00	160086	22653	1063
SSL	0.04 $\pm$ 0.01	0.05 $\pm$ 0.00	151870	24290	1550
EF	1.50	1.60	1.05	0.93	0.68

EF, enrichment factor (SML:SSL ratio).

**TABLE 3 | Summary of the net change (%) observed following aerosol addition ( $1.5 \text{ mg L}^{-1}$ ) in the SML and the SSL microcosms relative to unamended controls.**

Water sample	Time from aerosol addition (h)	BP	PP	LNA	HNA	<i>Synechococcus</i>	pico-eukaryotes	nano-eukaryotes
SML	1.5	126.8	12.2	1.7	87.6	0.6	18.4	3.6
	5	142.6	n.a	49.0	85.5	-1.3	1.1	-12.7
	9	108.9	n.a	16.8	49.2	-5.7	7.3	-15.6
	17	514.6	83.5	-6.8	36.2	-8.0	-7.3	-4.1
	21	643.1	48.6	7.4	40.9	-9.5	13.8	-2.1
	26	757.5	n.a	9.7	14.7	0.0	58.1	21.9
	44	503.3	83.1	20.7	101.8	3.7	87.7	16.0
SSL	1.5	67.3	2.0	-0.1	27.2	-7.8	25.4	-5.7
	5	51.3	n.a	12.6	23.2	-1.3	23.6	-0.5
	9	93.4	n.a	6.5	23.2	-1.9	4.2	-1.1
	17	131.4	72.5	-1.5	49.8	-1.1	8.4	7.7
	21	118.6	50.3	-1.0	31.1	2.2	-12.6	5.2
	26	174.7	n.a	5.6	38.8	-0.8	17.8	-1.1
	44	216.3	60.1	6.8	96.6	1.0	17.7	-3.4

n.a, not available.



in the control treatments and the C-rich monosaccharides concentration at the end of the experiment (T44), bacterial activity can account for 2.7 and 2.2  $\mu\text{g C L}^{-1}$  in the SML and SSL unamended microcosms, respectively. Simultaneously, the total consumption of [4,5-<sup>3</sup>H]-leucine (T44) in the SSL+aerosol can account for 5.4  $\mu\text{g C L}^{-1}$  and up to 15.7  $\mu\text{g C L}^{-1}$  in the SML+aerosol treatments. The concentration ( $\mu\text{g/Kg}$ ) of hydrocarbons delivered by the aerosol particles only was negligible relative to the ambient concentration in the water.

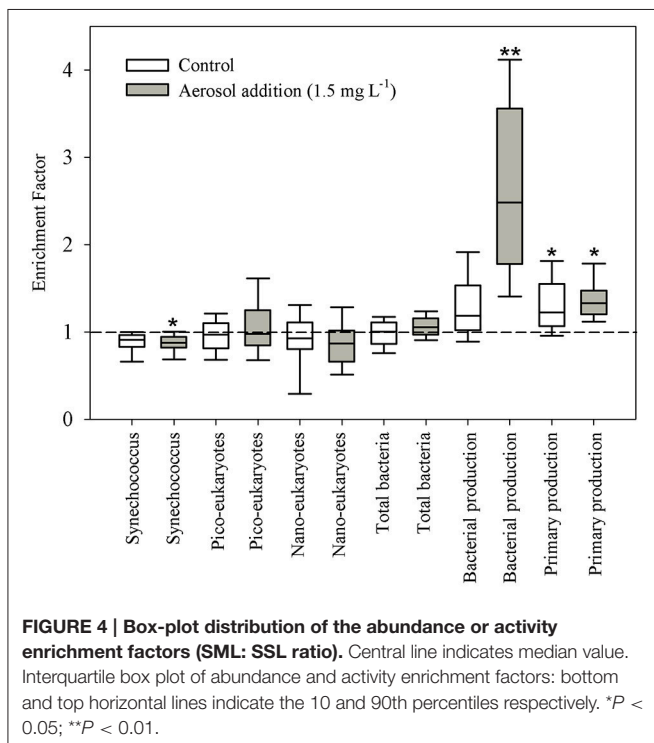
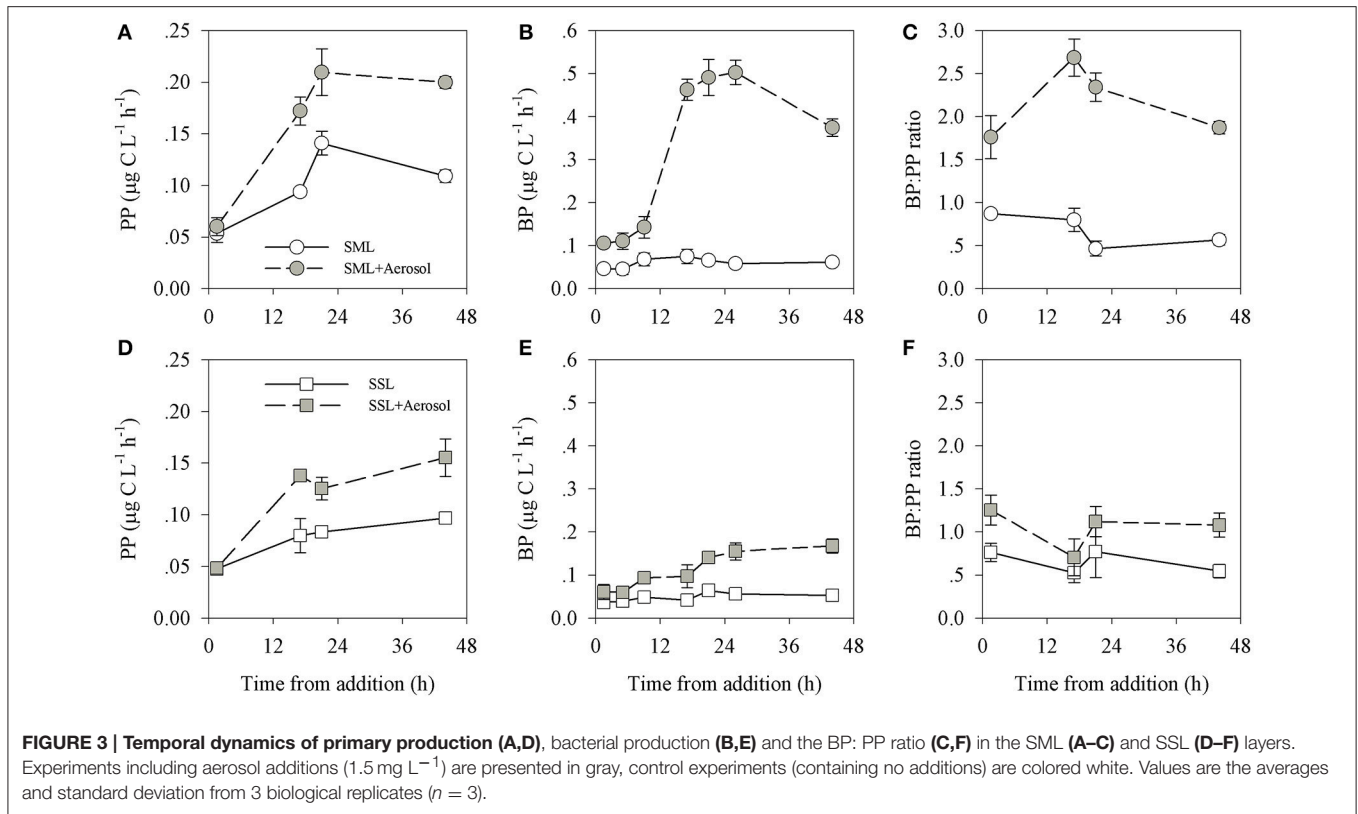
## DISCUSSION

Several studies have examined the effect of dust and aerosols on the marine bacterioplankton biomass and/or activity using model simulations (e.g., Mahowald, 2007; Guieu et al., 2014; Chien et al., 2016) and microcosm/mesocosm bioassays (e.g., Herut et al., 2005; Paytan et al., 2009; Marañón et al., 2010; Guieu et al., 2010; Romero et al., 2011; Rahav et al., 2016a). To

the best of our knowledge, none of these studies distinguished between the responses triggered by aerosol additions to the SML and those triggered by such additions to the SSL. In fact, these studies mostly considered the SSL (referred as “surface water” or “surface mixed layer”). Since the SML is the uppermost water layer that interacts with the atmosphere and to which atmospheric particles are directly deposited, its bacteria and plankton (neuston) inhabitants may respond differently than the SSL populations (Cunliffe et al., 2010; Vila-costa et al., 2013). Here we experimentally tested the response of these two water layers to natural dry deposition aerosol addition and compared the responses of the bacterioplankton communities in these two layers at the SEMs.

### SML Organic Enrichment

Our measurements demonstrate moderate, yet significant ( $P > 0.05$ ), differences in saccharides and hydrocarbons concentration between the SML and the SSL prior to the aerosol addition, resulting in  $\text{EF} > 1$  (Table 1). Thus, the SML was enriched in dissolved organic carbon, potentially bioavailable for bacterial



consumption, as observed in other marine environments (Cincinelli et al., 2001; Guigue et al., 2011; Santos et al., 2011). The major carbon source found in both layers was  $\alpha$ -olefins. These

hydrocarbons may originate from oil contamination (Riley et al., 1982), phytoplankton (Youngblood and Blumer, 1973) or even aerosol particles downwind to algae bloom (Ovadnevaite et al., 2011). It is possible that in the proximity to Haifa Bay industrial and rural area, aerosol particles, or water runoff could enrich the water with these hydrocarbons. Yet no other sign of such events was observed.

### SML Bacterioplankton Enrichment

Heterotrophic bacterial abundances prior to the aerosol addition were similar in the SML and in the underlying SSL water layer (EF  $\sim 1$ , Table 2). These results agree with several studies (Cunliffe et al., 2009, 2010), yet oppose others, which show significantly higher bacterial abundances in the SML (e.g., MacIntyre, 1974; Hardy, 1982). Picophytoplankton abundance at the SML was lower than at the SSL, especially the abundance of pico-eukaryotes (EF  $< 1$ ). Although studies reported an elevated phyto-neuston abundance relative to the SSL (e.g., Södergren, 1993; Guitart et al., 2013), it is apparent that several eukaryotic phytoplankton species, such as *Chaetoceros*, are less abundant in the SML (Hardy et al., 1984) or are differently affected by high light irradiation levels (Ruiz-González et al., 2012). A low autotrophic abundance in the SML might be explained by the intense radiation levels at this layer (particularly UV), which may cause photo-damage to the cells, especially in environments such as the SEMS in summer where radiation levels may reach  $>1000 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$  (Dishon et al., 2012).

## Activity in the SML and SSL

PP rates were higher at the SML relative to the SSL prior to and after the aerosol addition (Table 3, Figure 3). In contrast, BP rates were similar in both SML and SSL unamended microcosms and were elevated in both layers following aerosol additions, with a stronger response in the SML+aerosol microcosm. These results reflect higher cell-specific activity (production per cell) in the neuston communities that inhabit the SML relative to the bacterioplankton in the SSL. Relative to the very low concentration of inorganic nutrients characterizing this study area (Azov, 1986; Krom et al., 1991; Yacobi et al., 1995; Raveh et al., 2015), the addition of aerosol leached nutrients (as reported for this aerosol in Rahav et al. (2016b) would result in nearly doubling the levels of nitrate+nitrite (N, ~550 nM) and in a small increase in phosphorus concentration (P, ~12 nM). Assuming bacteria in the SEMS as P limited (Krom et al., 2010) and a 10–20 fg C per bacterial cell (Simon and Azam, 1989), the addition of P from the aerosol tested here (using a 106:1 Redfield C:P ratio), could explain an increase in bacterial abundance of up to  $\sim 1 \times 10^6$  cells<sup>-1</sup> mL<sup>-1</sup>, which is one order of magnitude higher than that measured for both the SML and SSL (net change of  $\sim 1 \times 10^5$  cells<sup>-1</sup> mL<sup>-1</sup>). It is possible that a great fraction of the added phosphorous was consumed by bacteria cells to fulfill their cellular metabolic needs rather than for growth/cell division. This is also exemplified in the increase in BP rates and the increase in %HNA bacteria following aerosol addition but not in the overall abundance of bacteria (Figures 2, 3). It may also suggest that the added P was taken by other compartments within the food-web (Thingstad et al., 2005; Pitta et al., 2016), or that P is not the limiting nutrient for bacterial activity as recently suggested (Tanaka et al., 2011; Rahav et al., 2016c). Alternatively, phosphorous might have been utilized faster by other microorganisms and transferred through the microbial food web directly to higher trophic levels (e.g., Thingstad et al., 2005), or that heterotrophic bacteria are not primarily limited by this element (e.g., Tanaka et al., 2011; Rahav et al., 2016c). Autotrophic microbes did not exhibit any significant increase in biomass, yet were more active as derived from the elevated PP rate (Figures 2, 3). Further, although relatively higher concentrations of hydrocarbons and saccharides were measured at the SML prior to the aerosol addition (as compared to the SSL), only slightly elevated BP or PP rates were recorded at the SML. Thus, the different carbon concentrations alone cannot explain the elevated BP and PP rates following the aerosol addition. These results are in agreement with a recent study performed in the same area showing a moderate increase in BP rates following the addition of glucose as a single amendment, while a significant enhancement in BP was reached after the addition of glucose, nitrogen and phosphate simultaneously (Rahav et al., 2016c). In addition to the aerosol derived N and P input, other micronutrients from the aerosols may also be limiting factors for microbial activity (e.g., Paytan et al., 2009). Tovar-sánchez et al. (2014) showed that Fe originating from aerosol particles was found to be unevenly distributed between the SML and SSL following a dust storm event. Thus, when considering the results described here and the work presented by Tovar-sánchez and colleagues, we assume that aerosol depositions

may trigger different impacts in these distinct layers. It is also likely that a high concentration of carbon, along with other aerosol-derived nutrients such as N and P or Fe that are added to a nutrient-poor aquatic system such as the SEMS, may not only alter the total microbial production, but also elevate the activity of specific groups in the SML. The difference in microbial production rates between the SML and SSL measured here are in agreement with a few studies that showed an equal or higher microbial activity in the SML relative to the SSL (e.g., Kuznetsova and Lee, 2001; Obernosterer et al., 2005), yet opposes other studies (Stolle et al., 2009; Santos et al., 2011; Sarmiento et al., 2015). It is well-known that diverse bacterial groups have different sensitivities to UV radiation (UVR). SAR11 activity, for example, is known to be inhibited by high UVR, whereas *Gammaproteobacteria* and *Bacteroidetes* show UVR resistance (Alonso-Sáez et al., 2007; Santos et al., 2011). Hence, it is likely that the unique community in the SML in the SEMS is well adapted to the high-light conditions in this environment and can take advantage of the higher carbon and nutrients concentration in the SML, thus grow faster than the SSL microbial communities despite the high radiation. We assume that the bulk microbial activity rate depends on the bacterial species relative abundance, their adaptation to the microenvironment and the availability of nutrients in the SML and SSL. The major reduction in dissolved n-alkanes and  $\alpha$ -olefins, measured 44 h after the aerosol addition in the SSL and SML, may be attributed to either heterotrophic consumption or adsorption by aerosol particles. So far only a few bacterial strains isolated from polluted aquatic habitats show high uptake rates of  $\alpha$ -olefins or alkanes on timescales similar to those examined here (Whyte et al., 1998). Thus, we estimate that in addition to possible heterotrophic utilization of these carbons, the major reduction in soluble alkanes and  $\alpha$ -olefins concentration was a result of adsorption to aerosol particles.

## Carbons Adsorption to Aerosol Particles

Contrary to alkanes and  $\alpha$ -olefins for which concentrations were almost completely reduced following aerosol amendment (T0 vs. T44h), monosaccharides decreased by only ~50–60% throughout the experiment's duration (Table 1). The monosaccharides' consumption (after 44 h) was almost the same in the SML and the SSL following aerosol additions (~420  $\mu\text{g L}^{-1}$ ), although different initial and end concentrations were recorded in both water layers (Table 1). Monosaccharides are considered as the energetically-favored carbon sources for heterotrophic bacterial metabolism (Kirchman, 2012), as opposed to alkanes and  $\alpha$ -olefins, which can be utilized only by a few specialized groups (Yakimov et al., 2007; Sevilla et al., 2015). Yet, based on the total leucine C incorporation during the experiment (derived from the BP measurements), less than 10% of the monosaccharides' reduction could be attributed to bacterial consumption in both water layers. We therefore assume that the physical adsorption of saccharides by the aerosol particles was more significant, and accounted for the remaining monosaccharides reduction. Relative to alkanes and  $\alpha$ -olefins, monosaccharides are more hydrophilic, we assume that as a result, these saccharides show a reduced adsorption



rate to the surface of the aerosol particles (“preferring” the water phase). Thus, unlike alkanes and  $\alpha$ -olefins that are more hydrophobic and were cleared from the soluble fraction following aerosol addition (primarily via adsorption), monosaccharides showed only a low reduction in concentration in the soluble fraction. Considering the abiotic removal via adsorption onto dust particles, a dust storm event such as the one examined here (September 2015), could substantially change the carbons availability to heterotrophic bacteria in the upper water layer and thus shift the PP to BP ratio from being “autotrophic-dominated” to “heterotrophic dominated.” Moreover, since considerable amounts of dust and aerosol are usually deposited in the SEMs (Herut et al., 1999, 2002), a chronic adsorption of various carbon substances to aerosol particles and their transfer down the water column may be a mechanism to transfer carbon to the deep water. The different types of carbon containing compounds (polar and hydrophobic) that were possibly adsorbed by the aerosol particles could later be leached off the particles at greater depths such as the deep chlorophyll maxima (usually 100–150 m in the SEMs, Kress et al., 2014) or even at the aphotic layer, thereby contributing to deep-water heterotrophic microbial activity. This adsorption may depend on aerosol concentration, composition, and timing of the deposition, and may thus have significant implications on the microbial loop. This mechanism may contribute to the relatively high metabolic activity of bacteria in the deep waters of the Mediterranean Sea attributed to the relatively warm conditions of this system (Luna et al., 2012). Further work should examine this mechanism of carbon transfer to the aphotic layers, as well as its availability to the subsurface microbial communities in the SEMs.

## CONCLUSIONS

We suggest that one of the major differences between the SML and SSL is the higher SML bacterioneuston activity rates that most probably resulted in the higher carbon utilization rate observed. It is possible that in this experiment, the consumption rate of monosaccharides differs between the SML and the SSL, although the same amount was consumed at the experiment end. Vila-costa et al. (2013), who studied freshwater SMLs bacteria and archaea communities following two Saharan dust storms, reported that the abundance of these groups did not change significantly following the dust storms. This finding is also supported by other studies in the Mediterranean (e.g., Tovar-sánchez et al., 2014). Yet, Vila Costa’s group reported that the composition of these groups was altered following aerosol introduction, resulting in a community shift (Vila-costa et al., 2013). In this study however, we did not characterize bacterial diversity (using molecular approaches such as 16S rRNA or 18S

rRNA) and thus we cannot determine whether community shifts occurred following aerosol additions. Nevertheless, the increase in bacterial activity in the SML following aerosol addition and the relative increase in HNA bacterial abundance (Table 3), may be an indication of the presence of bacterioneuston that are specifically adapted to take advantage of the constituents supplied by aerosols.

This study demonstrates the opportunistic character of the bacterioneuston community once nutrient-carrying airborne particles are introduced to the SML. Further, studies of the seasonal changes in the biodiversity and physiology of these communities in relation to atmospheric deposition from different sources are needed. Finally, the nature and dynamics of nutrients, metals (and microorganisms) exchange between the SML and SSL is currently unknown and warrants more study. In addition to carbon utilization, physical adsorption of these molecules by the aerosol particles may deliver carbon from the surface into deeper water, from the enriched SML to more oligotrophic layers of the sea.

## AUTHOR CONTRIBUTIONS

Conceived and designed the experiment: PA and ER. Performed the experiment: PA and ER. Analyzed the data: PA, AP, BH and ER. Contributed reagents/materials/analysis tools: PA and ER. Wrote the paper: PA, AP, BH and ER.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <http://journal.frontiersin.org/article/10.3389/fmars.2016.00222/full#supplementary-material>

**Supplementary Figure S1 | A three-day back trajectory analysis arriving at 100, 500, and 1000m altitude levels commencing at 10.00 UTC using the HYSPLIT (Hybrid Single-Particle Lagrangian Integrated Trajectory) model from the Air Resources Laboratory.** The star represents the study site off the SEMs.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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