Vol. 82: 31–42, 2018
https://doi.org/10.3354/ame01881

AQUATIC MICROBIAL ECOLOGY
Aquat Microb Ecol

Published online August 31

Response of active picoeukaryotes to the deposition of Saharan dust and European aerosols in the eastern Mediterranean Sea

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ABSTRACT: Atmospheric depositions are overwhelmingly recognized as being a driving force in marine ecosystems. However, little is known about the effect of atmospheric depositions on the active fraction of picoeukaryotic communities. Here, we reported the results of a mesocosm experiment which was designed to test the effects of Saharan dust (hereafter referred to as Dust) and European aerosols (hereafter called Aerosols) on planktonic communities in the eastern Mediterranean Sea (EMS). Pyrosequencing was performed (on Days 2 and 6 of a 9 d long incubation) for the V2–V3 region of 18S rRNA (from RNA that was reverse transcribed into cDNA) to determine community variations of active picoeukaryotes. Our results showed that Haptophyta represented the most active group on Days 2 and 6 (i.e. with relative abundances >78% in the Dust and Aerosols treatment groups as well as in the control), and they accounted for the major response of active picoeukaryotes to the addition of Dust and Aerosols (especially on Day 2). Our data also showed that the deposition of Dust and Aerosols differently influenced the composition of active picoeukaryotes, which included a number of unique taxa. In particular, the deposition of Dust led to a higher richness of active picoeukaryotes when compared with the deposition of Aerosols. In summary, our findings reveal the major influence of Dust and Aerosols on the community pattern of active picoeukaryotes in the EMS.

KEY WORDS: Mesocosm · Pyrosequencing · 18S rRNA · Atmospheric deposition · Haptophyta

INTRODUCTION

Atmospheric depositions are increasingly being recognized as a key driving force in shaping marine ecosystems. They are an important source of inorganic nutrients (Herut et al. 1999), trace metals (Bonnet & Guieu 2006, Westrich et al. 2016) and organic compounds (Kanakidou et al. 2012) to the ocean, and they can even affect pH (Loÿe-Pilot et al. 1986) and oxygen levels (Ito et al. 2016). For this reason, the impact of atmospheric deposition on the organisms in many marine ecosystems is well established. For instance, it was reported that a high-level supply of atmospheric deposition can relieve the phosphate (PO_4) and iron co-limitation of diazotrophy in the North Atlantic Ocean (Mills et al. 2004). In addition, the amount of atmospheric dust was shown to influence the coastal waters of the Mediterranean Sea and switch them from autotrophy to heterotrophy by altering bacterial production and community respiration (Lekunberri et al. 2010, Marín et al. 2017a,b). Furthermore, Asian dust was reported to remarkably

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enhance the growth of phytoplankton in the oligotrophic northern South China Sea (Guo et al. 2012, Wang et al. 2012).

However, the effect of atmospheric depositions on active picoeukaryotes has so far been rarely studied in marine ecosystems, although picoeukaryotes are important components of high diversity (Massana 2011, Caron et al. 2012). Relative to rDNA (rRNA gene)-based analysis, which provides a measure of viable organisms including both the active and inactive fractions, rRNA-based studies, which are derived from transcriptomics, are normally used to determine the active fraction of microbial communities (Stoeck et al. 2007, Massana et al. 2015). It is obviously more relevant to measure the active fraction of a microbial community, especially when attempting to clarify the biotic response to environmental variability (Jones & Lennon 2010, Blazewicz et al. 2013). For example, numerous rare but highly active marine picoeukaryotes were recently identified with rRNA sequencing (Koid et al. 2012, Logares et al. 2014), suggesting their ecological potentials for buffering environmental changes (Pedrós-Alió 2012).

The eastern Mediterranean Sea (EMS) is known to be an extremely oligotrophic region (Siokou-Frangou et al. 2010), with PO_4 limitation and a nitrate (NO_3) to PO_4 ratio in the deep waters of about 28:1 (Krom et al. 1991). In addition, the EMS is intensively influenced by 2 types of atmospheric deposition, dust from the Sahara (hereafter referred as Dust) and aerosols from Europe (hereafter referred as Aerosols) (Guerzoni et al. 1999). For example, Dust carried by storms supplies high levels of leachable inorganic nitrogen to the EMS (Carbo et al. 2005), and Aerosols have a major impact on the levels of black carbon and non-sea salt sulfate in Crete (located in the EMS) (Sciare et al. 2003). To investigate the effect of Dust and Aerosols depositions on EMS ecosystems, a mesocosm experiment was performed in the EMS (Herut et al. 2016). Subsequently, Tsagaraki et al. (2017) presented the overall response of the entire planktonic food web (from viruses to zooplankton) to the addition of Dust and Aerosols. Guo et al. (2016) and Tsiola et al. (2017) also reported that the bacterial abundance, production and growth rate were rapidly stimulated after the addition of Dust and Aerosols; however, Meador et al. (2017) reported that the bulk intact polar lipid biomarkers were not coupled with the stimulated response of plankton to the deposition of Dust and Aerosols. Christou et al. (2017) examined the feeding performance of the abundant calanoid copepod *Clausocalanus furcatus* but found no clear pattern of food selection. However, the addition of Dust and Aerosols did result in higher nitrogen (N_2) fixation rates (Rahav et al. 2016). The response of active picoeukaryotes to Dust and Aerosols has not yet been evaluated in this hot spot of intense interactions between atmospheric depositions and marine organisms.

In this study, we employed the mesocosm approach (Herut et al. 2016, Tsagaraki et al. 2017) to evaluate the response of active picoeukaryotes to Dust and Aerosols in the EMS. We performed 454 pyrosequencing for the V2–V3 region of picoeukaryotic 18S rRNA (from RNA that was reverse transcribed into cDNA) to target community compositions of active picoeukaryotes. Our aims were to (1) examine the response of active picoeukaryotes to atmospheric depositions and (2) compare the influences contributed by different types of atmospheric deposition.

MATERIALS AND METHODS

Mesocosm experiment setup

Enrichment experiments were performed using the CRETACOSMOS mesocosm facilities (www.cretacosmos.eu) of the Hellenic Centre for Marine Research (HCMR, Heraklion, Greece). Seawater was pumped from a 10 m depth into acid-cleaned high-density polyethylene containers at a station about 5 nautical miles north of Heraklion in the Cretan Sea (35° 24.975′ N, 25° 14.441′ E) on May 9 and 10, 2012 (Fig. 1). After collection, the seawater was transported to the HCMR within 2 h and evenly distributed to fill 9 food-grade polyethylene mesocosm bags

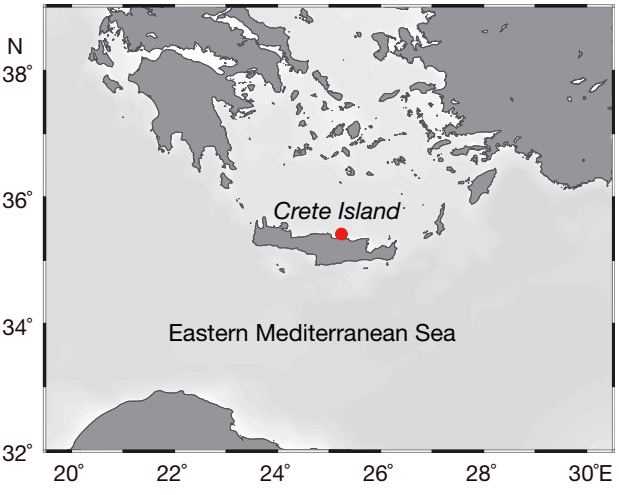

Fig. 1. Sampling location (red dot), about 5 nautical miles north of Heraklion (Crete, Greece)

to a final volume of ~2.8 to 3 m^3. These bags were immersed in a concrete tank of 350 m^3 and incubated at an *in situ* temperature of ~19.5°C. To avoid stratification in the mesocosm bags, air was bubbled with an airlift system, ensuring a general mixing of the water column. In addition, a Plexiglas lid was used to protect the mesocosm bags from atmospheric deposition, and a mesh screen was attached on the lid to mimic the lighting conditions at the sampling depth.

The Dust used in the experiments was collected from locations in Crete (Heraklion and Sambas) and Israel (Beit Yannay and Haifa) during Saharan dust storms (i.e. May 2007 and April 2012), and Aerosols (i.e. suspended particles in the air) were collected during periods of air mass transport from Europe (i.e. in April 2011 and during April and May 2012) as described by Herut et al. (2002). Following dust storms, samples were collected using a glass panel collector or a plastic surrogate collector, and the powder was scraped from the collectors using a plastic knife into acid-cleaned 15 ml scintillation vials. Aerosols were collected using Whatman filters and a high-volume sampler at a flow rate of 42 m^3 h^{-1} for 19 to 114 h, after which the filters were dried in a desiccator for 24 h. To collect sufficient amounts of Dust and Aerosols, the deposited materials sampled on different dates were pooled. In addition, a limited amount of Aerosols were collected to represent European aerosol pollution. To determine the composition of Dust and Aerosols, leaching experiments were performed according to methodologies described in detail by Herut et al. (2016).

Two experimental amendments (i.e. Dust and Aerosols treatments) were made in addition to the untreated control using the 9 mesocosms (i.e. each treatment was conducted in triplicate). The Dust and Aerosols (both collected in Crete and Israel) were separately mixed with filtered (0.2 µm) seawater and added at a final concentration of 1.6 and 1 mg l^{-1}, respectively (Table 1). The control treatment, without the addition of Dust or Aerosols, was run in parallel.

Table 1. Amounts of Saharan dust (Dust) and European aerosols (Aerosols) added in the mesocosm experiments. The leachable concentrations of nitrite + nitrate ($NO_2 + NO_3$) and phosphate (PO_4) derived from these additions are shown; the estimations of the leachable nutrient concentrations are calculated as described in Herut et al. (2016)

Addition	Concentration (mg l^{-1})	$NO_2 + NO_3$ (nM)	PO_4 (nM)
Dust	1.6	36.8	3.9
Aerosols	1	54	3

The mesocosm experiments ran for 9 d (with Day 1 being the day that the Dust or Aerosols treatment began). A more detailed description of the mesocosm protocol used can be found in Herut et al. (2016) and Tsagaraki et al. (2017).

Sampling collection

RNA samples were collected on Days 2 and 6 from each mesocosm. A total of 9 to 10 l seawater was prefiltered through GF/D filters (2.7 µm pore size and 47 mm diameter; Whatman) within ~40 min. The prefiltered seawater was then filtered through 0.2 µm pore size polyethersulfone filters (Pall) using a peristaltic pump. The polyethersulfone filters were immediately immersed into RNAlater (Ambion) in RNAase-free tubes and stored at −80°C.

Daily samplings prior to (Day 0) and at Days 1 to 8 following the addition of Dust/Aerosols were also conducted to determine a number of parameters, including chl *a*, primary productivity, pigmented picoeukaryote abundance and nutrient concentration. Chl *a* and primary productivity were analyzed at 3 size fractions (i.e. 0.2–0.6, 0.6–2.0 and >2.0 µm), and the contribution of pico-sized organisms was calculated using the sum of the measurements of the 0.2–0.6 and 0.6–2.0 µm fractions (i.e. 0.2–2.0 µm). The methods used to obtain these estimates have been described in detail by Tsagaraki et al. (2017). However, in brief, the concentration of chl *a* concentrations was determined fluorometrically (Holm-Hansen et al. 1965), the primary production rate was calculated using the ^{14}C incorporation method (Steemann Nielsen 1965), and the abundance of picoeukaryotes was estimated using a FACSCalibur (Becton Dickinson) flow cytometer (Marie et al. 1999). In addition, the concentrations of nitrite (NO_2), NO_3 and silicate (SiO_3) were measured by methods described by Strickland & Parsons (1972), while that of ammonium (NH_4) was measured according to Ivančič & Degobbis (1984), and that of PO_4 was measured using the MAGIC method, as described by Rimmelin & Moutin (2005).

RNA extraction and cDNA synthesis

Total RNA was extracted using the TRIzol plus RNA Purification Kit (Invitrogen). First, the RNAlater was removed from the filters. The filters were then incubated in 1 ml TRIzol reagent for 5 min at room temperature, after which 200 µl chloroform was

added and incubated for 3 min at room temperature, with thorough shaking. The samples were then centrifuged for 15 min at 12 000 × *g* and 4°C, and the colorless upper aqueous phase containing the RNA was transferred to a new tube. The RNA was precipitated with an equal volume of 70% ethanol and transferred to PureLink RNA Mini Kit columns, after which the purification, washing and elution of RNA was performed following the manufacturer's instructions. The amount of RNA extracted was measured with a NanoDrop 1000 Spectrophotometer (Thermo Scientific).

The total RNA was reverse transcribed to cDNA using the SuperScript III First-Strand Synthesis System (Invitrogen) according to the manufacturer's instructions. Prior to the cDNA synthesis, a DNase I (Invitrogen) treatment was performed to remove residual DNA. The transcribed cDNA from triplicate mesocosms was pooled together for further processing.

454 pyrosequencing and sequence processing

The V2–V3 region of 18S rRNA in the cDNA products was amplified to determine the community composition of active picoeukaryotes. PCR was performed using a pair of 454 fusion primers of 18S-82F (5′-GAA ACT GCG AAT GGC TC-3′) (López-García et al. 2003) and Euk516r (5′-ACC AGA CTT GCC CTC C-3′) (Díez et al. 2001), and the amplification mix contained 1 unit *Taq* polymerase (Invitrogen), 1× reaction buffer, 0.2 mM dNTPs, 1 mM magnesium chloride and a 0.2 µM concentration of each primer. Multiple identifier (MID) tags of 10 bp were assigned to the forward primer to identify amplicons belonging to the different samples. The PCR program consisted of an initial denaturation step at 95°C for 2 min, followed by 35 cycles of 95°C for 30 s, 55°C for 30 s and 72°C for 40 s and a final extension at 72°C for 7 min. Triplicate PCR products of each sample were pooled and purified using the Gel Band Purification Kit (GE Healthcare), and the purified products were quantified with the Quant-iT picoGreen dsDNA Assay Kit (Invitrogen). Equal amounts of the MID adaptor-labeled PCR products were mixed, and emPCR was carried out according to the 454 rapid library construction protocol (Roche). Pyrosequencing was performed on a GS Junior System (Roche). The raw sequences have been deposited in the National Center for Biotechnology Information Sequences Read Archive under accession number PRJNA414354.

Sequence processing was conducted using the Quantitative Insights Into Microbial Ecology (QIIME v. 1.9.1) pipeline (Caporaso et al. 2010). Sequences with a length of 150 to 500 bp and quality scores >25 were kept, but no primer mismatches or ambiguous base calls were kept. Suspected chimeras were identified and removed using ChimerSlayer (Haas et al. 2011). Operational taxonomic units (OTUs) were grouped using the sumaclust algorithm (Mercier et al. 2013) at 97% similarity, and a representative set of sequences was generated and assigned using BLAST (E value = 10^{-6}) against the Protist Ribosomal Reference database (PR^2) (Guillou et al. 2013). OTUs represented by 1 single read (singletons) and OTUs assigned to Metazoa were discarded from the downstream analysis.

Alpha diversity indices (Chao1 and ACE) for each sample were calculated using the vegan package (Oksanen et al. 2014) in the R program (R Core Team 2014). Venn diagrams were generated to depict numbers of shared and non-shared OTUs among the control, Dust and Aerosols (on Days 2 and 6). Beta diversity indices (Bray-Curtis dissimilarity) among samples were also estimated using the vegan package.

Phylogenetic analysis

To provide phylogenetic perspectives into the key taxa (defined as OTUs present in all samples), we constructed a phylogenetic tree for a subset of key OTUs (successfully detected across 6 samples) affiliated to the dominant group (Haptophyta). In brief, representative key OTUs of Haptophyta were aligned using MAFFT (Katoh & Standley 2013) after adding reference sequences. A maximum-likelihood phylogenetic tree was constructed using PhyML (Guindon et al. 2010) (1000 bootstrap replicates) with the TrNef + G model, as proposed by jModeltest (Darriba et al. 2012) under Akaike's information criterion. In addition, the relative abundances of these key OTUs in each whole community were visualized by a heatmap.

RESULTS

Chemical compositions of depositions and leached nutrients

When comparing the composition of Dust and Aerosols, the majority of the element concentrations displayed similar values (Table S1 in the Supplement at www.int-res.com/articles/suppl/a082p031_supp.pdf). However, a few trace metals (e.g. copper, lead, zinc and cadmium) were enriched in Aerosols (i.e. with val-

ues of 59, 23, 190 and 0.32 ppm, respectively) relative to Dust (with values of 47, 19, 162 and 0.21 ppm, respectively) (Table S1). Details of the leached nutrients have already been described by Herut et al. (2016). However, in brief, the Dust and Aerosols treatments received, respectively, 36.8 and 54 nM for $NO_2 + NO_3$ and 3.9 and 3.0 nM for PO_4 (Table 1).

Hydrographic conditions

The ambient seawater used for setting up the mesocosms was typically ultra-oligotrophic, showing a chl *a* concentration of 0.064 µg l^{-1} and primary production rate of 0.39 ± 0.02 (mean ± SD) µg C l^{-1} h^{-1}. These values are at the lower end of the range of oligotrophic systems in the world oceans (Herut et al. 2016).

During the 9 d incubation, picophytoplankton chl *a* generally exhibited higher concentrations in the Dust and Aerosols treatment groups than in the control (Fig. 2A). Similar values were obtained for enrichment with both Dust and Aerosols on both Day 2 (Dust, 0.07 ± 0.001 µg l^{-1}; Aerosols, 0.08 ± 0.002 µg l^{-1}) and Day 6 (Dust, 0.05 ± 0.003 µg l^{-1}; Aerosols, 0.06 ± 0.001 µg l^{-1}). In addition, when accumulating data for the entire incubation period, picophytoplankton accounted for average fractions at a similar range to the total chl *a* concentrations in the control (61 ± 2.8%), Dust (57.1 ± 4.3%) and Aerosols (60.3 ± 2.1%) treatments (Fig. S2A in the Supplement), with the control and Dust data alone being statistically significantly different (at $p < 0.05$, *t*-test). However, for the relative contributions of picophytoplankton to the total primary production rates, the Aerosols treatment group (45.4 ± 5.6%) was significantly ($p < 0.05$, *t*-test) higher than the control (31.6 ± 10%), but no significant differences ($p > 0.05$, *t*-test) were found when comparing the Dust treatment group (37 ± 11.5%) with the control (Fig. S2B). More specifically, picophytoplankton primary production rates in the Dust and Aerosols treatments were higher than in the control on Day 2 ($p < 0.05$ for each comparison, *t*-test) (control, 0.08 ± 0.04 µg C l^{-1} h^{-1}; Dust, 0.25 ± 0.04 µg C l^{-1} h^{-1}; Aerosols, 0.31 ± 0.07 µg C l^{-1} h^{-1}) but not on Day 6 ($p > 0.05$ for each comparison, *t*-test) (control, 0.11 ± 0.04 µg C l^{-1} h^{-1}; Dust, 0.17 ± 0.04 µg C l^{-1} h^{-1}; Aerosols, 0.2 ± 0.07 µg C l^{-1} h^{-1}) (Fig. 2B). Moreover, the cell counts of photosynthetic picoeukaryotes in Dust and Aerosols were not significantly different ($p > 0.05$, *t*-test) on both Day 2 (Dust, 2193 ± 52 cells ml^{-1}; Aerosols, 2148 ± 98 cells ml^{-1}) and Day 6 (Dust, 1025 ± 66 cells ml^{-1}; Aerosols, 987 ± 74 cells ml^{-1}). These cell numbers for both Dust and Aerosols were not significantly different ($p > 0.05$, *t*-test) from that of the control on Day 2 (1981 ± 207 cells ml^{-1}), but they were significantly higher than that of the control on Day 6 (784 ± 26 cells ml^{-1}) ($p <$

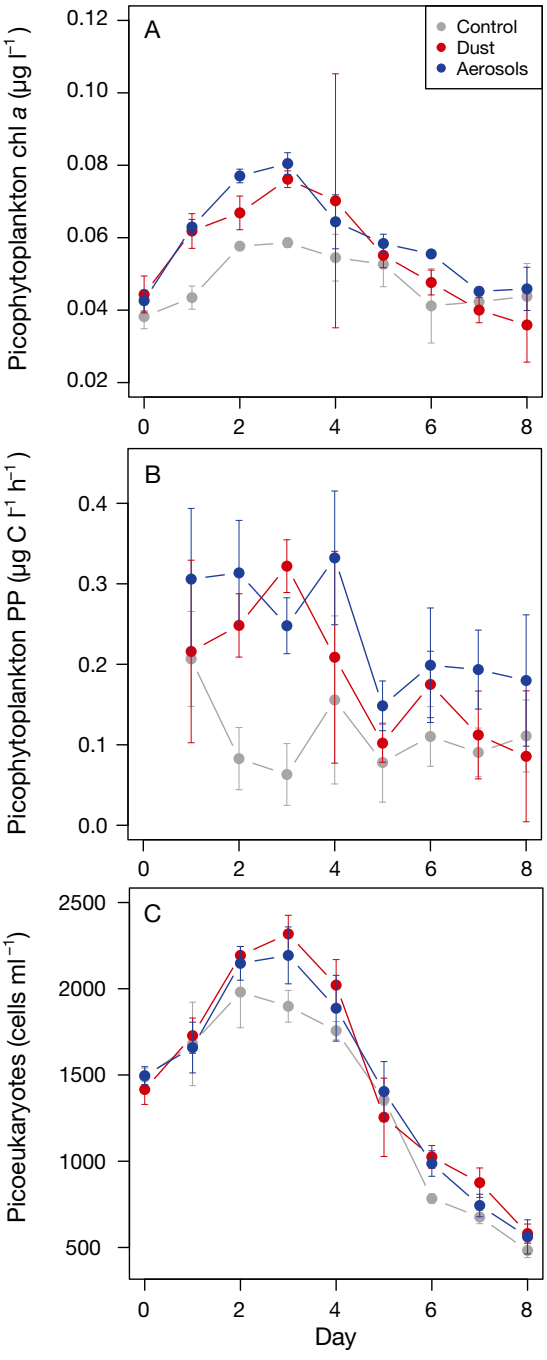

Fig. 2. Concentration of (A) picophytoplankton chl *a*, (B) picophytoplankton primary production (PP) and (C) pigmented picoeukaryote counts throughout the mesocosm experiments. Values are mean ± SD of 3 replicates. Dust: Saharan dust; Aerosols: European aerosols

0.05, *t*-test) (Fig. 2C). The nutrients (NH_4, NO_3, PO_4 and SiO_3) generally showed higher concentrations under the Dust and Aerosols treatments during the incubation period, in spite of great variations in the estimations (Fig. S2 in the Supplement).

Pyrosequencing characteristics

A total of 16 694 high-quality reads (with 2377–3098 reads per sample) (Table S2) were generated, with an average length of 301 bp. The OTU numbers for the 6 samples ranged from 98 (in the Day 6 control) to 137 (in the Day 2 Dust treatment group). In addition, the Chao1 and ACE indices showed the lowest and highest values for the Day 6 control group (with Chao1 = 136 and ACE = 142) and the Day 2 Dust group (with Chao1 = 226 and ACE = 246), respectively (Table S2). Among the retrieved OTUs, 35.4% (i.e. 70 out of 198) and 35.7% (i.e. 61 out of 171) were shared in the Day 2 and Day 6 samples, respectively (Fig. 3A,B).

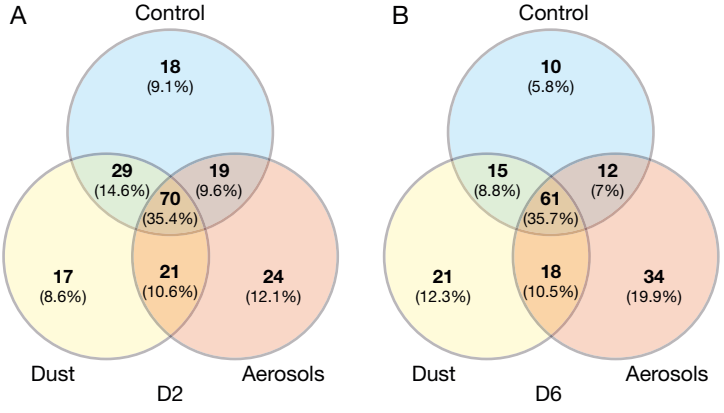

Fig. 3. Venn diagrams showing the number of operational taxonomic units shared by, and unique to, each treatment on (A) Day 2 (D2) and (B) Day 6 (D6). Dust: Saharan dust; Aerosols; European aerosols

Community patterns

The active fractions of picoeukaryotic communities were dominated by Haptophyta, showing an average relative abundance of 82.3% on Day 2 and 86.6% on Day 6 (Fig. 4). More importantly, the Haptophyta fraction exhibited the greatest difference in relative abundance on Day 2. In addition, on Day 2 of treatment with Dust or Aerosols, the contribution of Haptophyta increased by 6.8 and 6.0%, respectively, relative to the control, whereas the contribution of Cercozoa decreased by 4.4 and 4.6%, respectively (Fig. 4A). In contrast, on Day 6 of treatment, Haptophyta contributed less in the Dust (85.9%) and Aerosols (84.5%) treatment groups when compared with the control

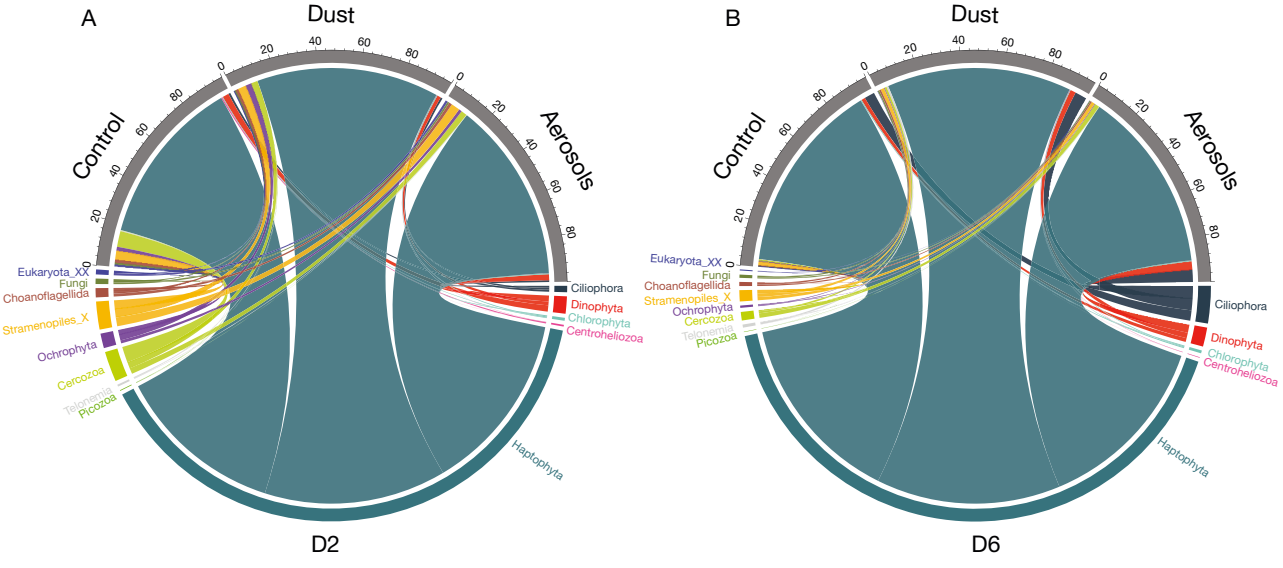

Fig. 4. Taxonomic affiliations (roughly at the phylum level) of active picoeukaryotic 18S rRNA sequences on (A) Day 2 (D2) and (B) Day 6 (D6). The relative abundance (%) of sequences that belonged to each taxonomic group within a sample is represented by the thickness of the colored area and calculated by the outer grey histograms (upper half). The lower half of the outer (colored) histogram represents the relative abundance of each taxonomic group in the whole 3 samples. Dust: Saharan dust; Aerosols: European aerosols

(89.3%) (Fig. 4B), whereas relative to the control, Cercozoa (Dust, 0.4%; Aerosols, 1.5%), Dinophyta (Dust, 0.7%; Aerosols, 2.1%) and Ciliophora (Dust, 0.8%; Aerosols, 0.9%) all exhibited an increased contribution in the Dust and Aerosols treatment groups. Moreover, comparing the community patterns on Days 2 and 6, Haptophyta, Ciliophora, Dinophyta and Telonemia all displayed higher relative abundances on Day 6 than on Day 2 with increases of 4.3, 4.5, 0.3 and 0.2%, respectively, whereas the other groups exhibited decreased relative abundances.

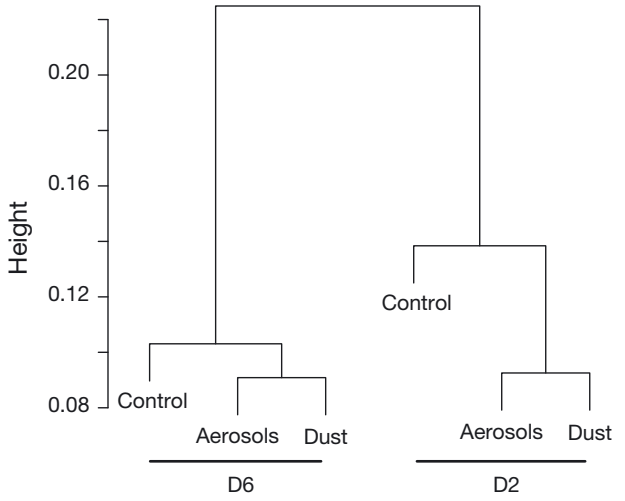

Fig. 5. Unweighted pair group method with arithmetic mean clustering of active picoeukaryotic communities based on the Bray-Curtis dissimilarity. Dust: Saharan dust; Aerosols: European aerosols; D2: Day 2; D6: Day 6

Based on these relative abundance data, the communities in the Dust and Aerosols treatment groups were remarkably dissimilar to the control (Fig. 5). In addition, more differences were observed on Day 2 than on Day 6 when comparing the deposition-enriched treatments with the control.

Distribution variations of detected Haptophyta orders (roughly at rank 5 in PR^2) exhibited several remarkable responses to the different treatments at a finer taxonomic level (Fig. 6). For example, when comparing the Dust and Aerosols treatments relative to the control on Day 2, they resulted in higher contributions of Phaeocystales (i.e. Dust–control, 4.2%; Aerosols–control, 5.1%) and Prymnesiales (i.e. Dust–control, 1.9%; Aerosols–control, 1.5%) but lower

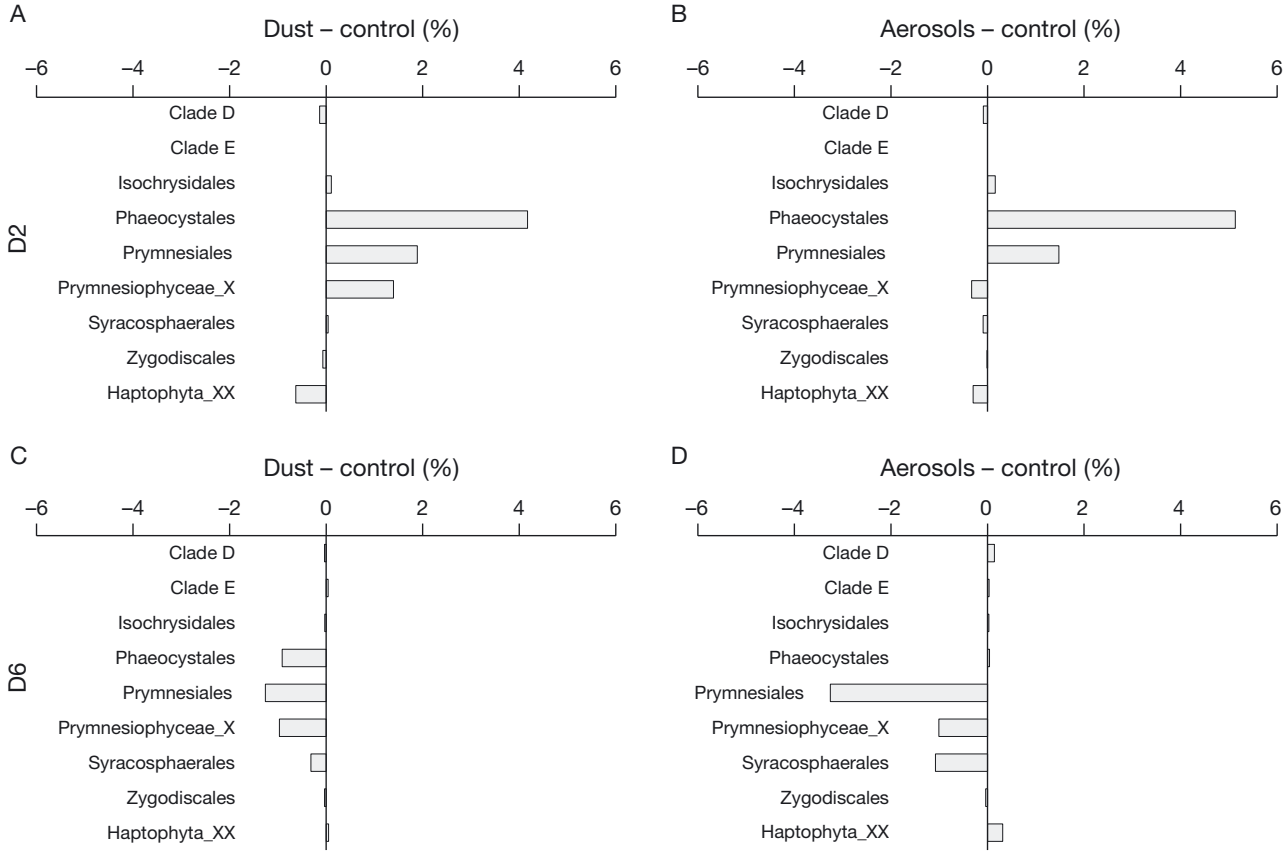

Fig. 6. Differences in the relative abundance of Haptophyta sub-groups between atmospheric depositions and the control on (A,B) Day 2 (D2) and (C,D) Day 6 (D6). Dust: Saharan dust; Aerosols: European aerosols

contributions of Haptophyta_XX (i.e. Dust–control, –0.6%; Aerosols–control, –0.3%) (Fig. 6A,B). Contrasting outcomes were observed for the order Prymnesiophyceae_X, which showed an increase in the Dust treatment (Dust–control, 1.4%) but a decrease in the Aerosols treatment (Aerosols–control, –0.3%) on Day 2. In contrast to Day 2, on Day 6, Phaeocystales showed a lower contribution (i.e. of –0.9%) in the Dust treatment group when compared with the control but displayed only a very slight difference (<0.1%) in the Aerosols treatment group (Fig. 6C,D). In addition, Prymnesiales, Prymnesiophyceae_X and Syracosphaerales also all displayed lower relative contributions in the Dust and Aerosols treatments when compared with the control.

Phylogenetic analyses of the key OTUs within Haptophyta

A total of 18 key OTUs affiliated to Haptophyta were observed; these were present across all 6 of the samples and belonged to 6 orders (Fig. 7). OTU2 was affiliated to Prymnesiales and represented on average

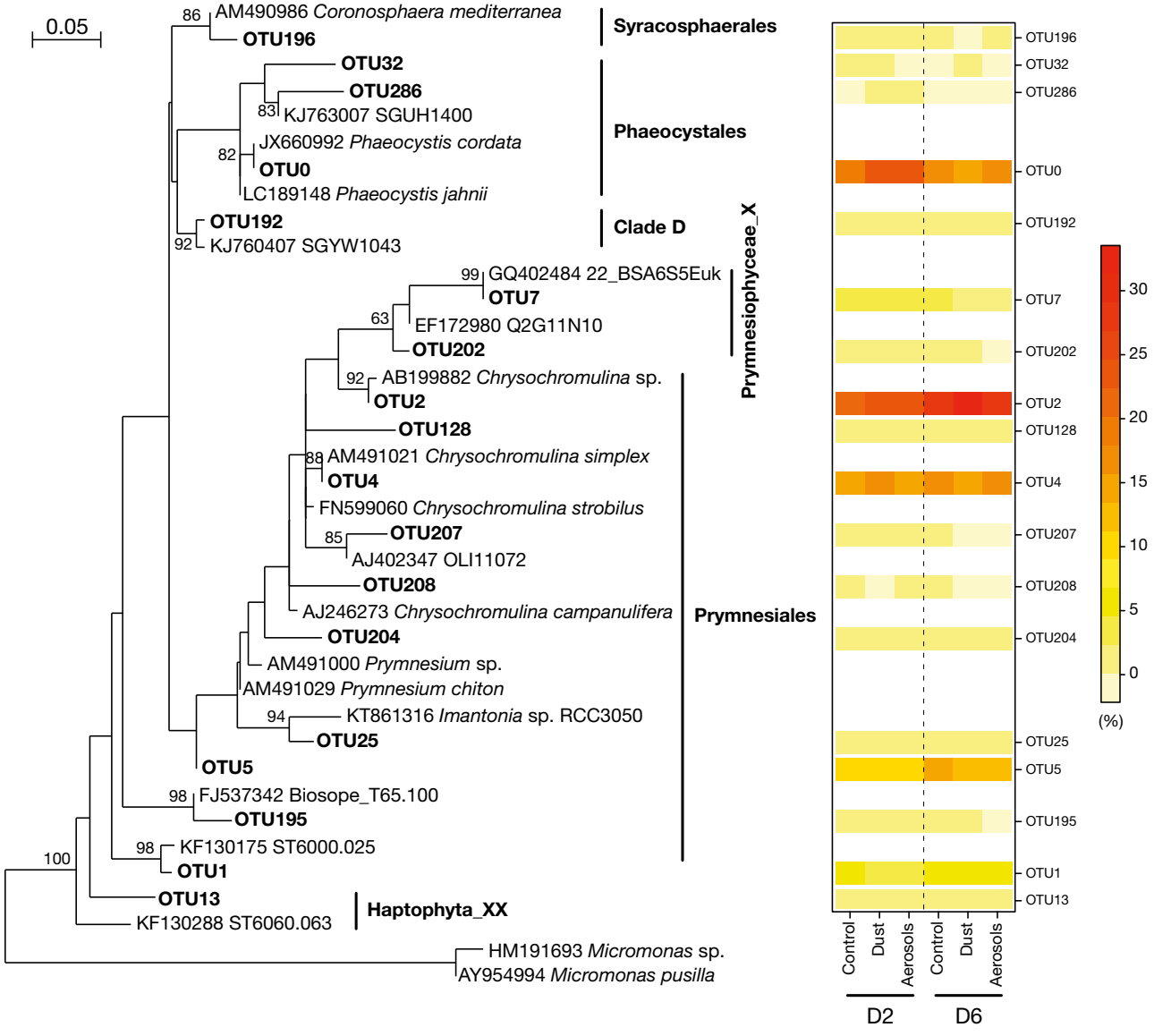

Fig. 7. Heatmap-combined maximum-likelihood tree of 18S rRNA for the 18 key Haptophyta operational taxonomic units (OTUs). The tree is rooted using 2 *Micromonas* sequences. The scale bar indicates 0.05 substitutions per base. The color bar of the heatmap represents the relative abundance of each OTU in active picoeukaryotic communities. Dust: Saharan dust; Aerosols: European aerosols; D2: Day 2; D6: Day 6

~26% of the sequences in the 6 communities. OTU0 (Phaeocystales), OTU4 (Prymnesiales) and OTU5 (Prymnesiales) also showed remarkable contributions, comprising ~19.2, ~15.4 and ~11.4%, respectively, of the sequence. In addition to abundant OTUs belonging to Phaeocystales and Prymnesiales, various other OTUs, including OTU196 (0.2%; Syracosphaerales), OTU7 and OTU202 (2.5 and 0.1%, respectively; Prymnesiophyceae_X), OTU192 (0.4%; Clade D) and OTU13 (0.9%; Haptophyta_X), were present with relatively low average contributions.

DISCUSSION

Responses of active picoeukaryotes

Our results showed that Dust and Aerosols triggered substantial changes in the composition of the active picoeukaryotic communities in the EMS. Atmospheric depositions are important in ultra-oligotrophic regions such as the EMS where new nutrients are only rarely delivered from other external sources (Herut et al. 1999, Krom et al. 2004, Duce et al. 2008). In previous mesocosm experiments, it was reported that Dust and Aerosols elicited positive changes in the abundance of bacteria as well as bacterial production, phytoplankton growth and primary production (Gallisai et al. 2014, 2016, Herut et al. 2016). Our new results provide additional evidence regarding the impact of Dust and Aerosols on active picoeukaryotes in the EMS; they demonstrate that different active picoeukaryotes display distinct responses to the deposition of Dust and Aerosols.

Haptophyta are the main active picoeukaryotes that respond to the deposition of Dust and Aerosols, both in terms of their dominance and the changes in their relative abundance within the picoeukaryotic community. The dominance of Haptophyta (particularly Prymnesiophyceae) in all the treatment groups (Fig. 4) was consistent with previous findings that Haptophyta are major eukaryotic producers and are extremely diverse in the open oceans (Liu et al. 2009). Haptophyta are also reported to dominate the photosynthetic picoeukaryotic communities especially in extremely oligotrophic regions, as revealed by photosynthetic pigment analysis (Peloquin et al. 2013), plastid 16S rDNA sequencing (Shi et al. 2011) and 18S rDNA sequencing of flow cytometry-sorted samples (Shi et al. 2009). This surplus of Haptophyta might occur because of a few special ecological strategies. We suggest that among the photosynthetic picoeukaryotes, they might represent the dominant group in the *in situ* active community fraction (Marquardt et al. 2016) in the surface EMS (Man-Aharonovich et al. 2010). In particular, Haptophyta might contribute the largest fractions of chl *a* biomass and primary production of the photosynthetic picoeukaryotes (Fig. 2A,B), with both being stimulated by the addition of Dust and Aerosols. Our findings are supported by their reported relative abundances in active picoeukaryotic communities (Giner et al. 2016). However, the relative contributions we obtained were much higher than in many of the *in situ* communities without incubation (Massana et al. 2015); this phenomenon might have occurred because of the tank enclosure (Countway et al. 2005, Massana et al. 2006, Kim et al. 2011).

The evident responses of Haptophyta at high (phylum) (Fig. 6) and low (OTU) (Fig. 7) taxonomic levels might be due to a few special ecological strategies. The majority of Haptophyta are known to use the crucial ecological strategy of mixotrophy, which facilitates the tolerance of oligotrophic conditions in open oceans (Zubkov & Tarran 2008, Unrein et al. 2014). The increasing relative abundance of Haptophyta observed in the Dust and Aerosols treatment groups might also be due to the rapidly increasing availability of prey bacteria (Guo et al. 2016). Moreover, Haptophyta have recently been found harboring N_2-fixing unicellular cyanobacteria group A (UCYN-A) (Thompson et al. 2012, Hagino et al. 2013). Indeed, it has been suggested that there is a symbiotic relationship between Haptophyta and UCYN-A, such that the former provide carbon compounds for the latter and, in return, they obtain nitrogen compounds (Thompson et al. 2012). Although UCYN-A was previously found in the western Mediterranean Sea (Le Moal et al. 2011), it was not detected during mesocosm sampling based on 16S rDNA and *nifH* gene sequencing (Guo et al. 2016, Rahav et al. 2016). This suggests that any symbiotic relationship with UCYN-A in this study might be a minor strategy for Haptophyta. In addition, the Haptophyta might be directly stimulated by the addition of Dust and Aerosols, which have been shown to supply critical elements such as nutrients (Fig. S1 in the Supplement at www.int-res.com/articles/suppl/a082p031_supp.pdf) and trace metals (Herut et al. 2016) for photosynthesis, which in turn can result in a higher relative abundance (Fig. 6).

Different phylotypes (particularly the key OTUs) within Haptophyta exhibited distinct responses to the atmospheric deposition of Dust and Aerosols (Fig. 7). This resulted in the aforementioned changes in the relative contributions at the phylum level.

For example, of the most abundant OTUs, OTU2 accounted for higher relative contributions (in particular on Day 2) in the Dust and Aerosols treatment groups. This suggests that *Chrysochromulina* (Prymnesiales) might benefit rapidly from the addition of Dust and Aerosols. Similarly, the increased contributions of OTU0 (again mainly on Day 2) indicated that *Phaeocystis* (Phaeocystales) also had a positive response to the addition of Dust and Aerosols regarding their relative abundance. In contrast, negative responses were observed for OTU0 on Day 6 in the Dust treatment (Figs. 6C & 7). Negative responses were also found for OTU5 (Prymnesiales) (Fig. 7), which contributed most of the responses at the genera level (Fig. 6C,D). We speculate that these species (OTUs) had a better ability (than groups such as *Chrysochromulina* and *Phaeocystis*) to adapt to the extreme oligotrophic environment of the control, and thus they showed negative responses with Dust and Aerosols on Day 6, when the levels of enriched nutrients were nearly exhausted.

Differing effects of Dust and Aerosols

The deposition of Dust and Aerosols triggered different responses on the active picoeukaryotic communities. The Dust and Aerosols treatment groups contained a number of unique OTUs on Day 2 (i.e. 8.6% for Dust and 12.1% for Aerosols) and Day 6 (12.3% for Dust and 19.9% for Aerosols) (Fig. 3B,C), indicating that the chemical components of each could differently enhance distinct species. For example, the Dust treatment benefitted more unique Haptophyta (Fig. S3B,C in the Supplement) and Chlorophyta (Fig. S3E,F) and supported higher diversity (Chao1 and ACE estimates) than did the Aerosols treatment (Table S2). This suggests that the chemical composition of this type of Dust (Herut et al. 2016) supported the growth of rare species more than that of the Aerosols. The different responses of active picoeukaryotes to the Dust and Aerosols treatments might therefore be attributed to their distinct chemical compositions (Table S1). For example, the Dust contained larger amounts of mineral components, whereas the Aerosols consisted of more anthropogenic components. Moreover, the differential effects of Dust and Aerosols on the bacterial communities have previously been reported to be due to the contrasting amounts of enriched substrates (Cornell et al. 1995, Pulido-Villena et al. 2008). Overall, our results indicate that different deposition events in the EMS have distinct effects on the active picoeukaryotic communities, as has previously been reported for the bacterial communities (Gallisai et al. 2016, Guo et al. 2016, Marín et al. 2017a,b, Tsiola et al. 2017).

In addition, the community successions that were observed from Days 2 to 6 suggest a dynamic response of the active picoeukaryotes, as a result of the deposition of both Dust and Aerosols. In particular, for subgroups of Haptophyta, contrasting differences in relative contributions were observed on Days 2 and 6, when compared with the control (Fig. 6). In addition, even though only relatively small variations occurred for Haptophyta between Days 2 and 6, these successions indicate that the underlying biogeochemical dynamics of atmospheric components happen at a finer scale.

In summary, we used mesocosm experiments to evaluate the response of active picoeukaryotes to the addition of Dust and Aerosols in the EMS. We found that Haptophyta strongly dominated the community patterns across the control and enrichment groups and displayed corresponding changes at different taxonomic levels. The Dust and Aerosols treatments also had different effects on the active picoeukaryotic communities. Our results provide new evidence, which helps with our understanding of how atmospheric depositions influence marine ecosystems.

Acknowledgements. This study was supported by the EU-FP7 project MESOAQUA: a network of leading MESOcosm facilities to advance the studies of future AQUAtic ecosystems from the Arctic to the Mediterranean. We also acknowledge support from the Hong Kong Research Grants Council via General Research Funds (GRF 661912, 661813 and 16128416). The authors thank G. Piperakis for technical assistance throughout this project and both S. Zivanovic and E. Dafnomili for help with chemical analyses. We also thank the captain and crew of the RV 'Philia' for their assistance with the transportation of seawater.

LITERATURE CITED

Blazewicz SJ, Barnard RL, Daly RA, Firestone MK (2013) Evaluating rRNA as an indicator of microbial activity in environmental communities: limitations and uses. ISME J 7:2061–2068

Bonnet S, Guieu C (2006) Atmospheric forcing on the annual iron cycle in the western Mediterranean Sea: a 1-year survey. J Geophys Res 111:C09010

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K and others (2010) QIIME allows analysis of high-throughput community sequencing data. Nat Methods 7:335–336

Carbo P, Krom MD, Homoky WB, Benning LG, Herut B (2005) Impact of atmospheric deposition on N and P geochemistry in the southeastern Levantine basin. Deep-Sea Res II 52:3041–3053

Caron DA, Countway PD, Jones AC, Kim DY, Schnetzer A (2012) Marine protistan diversity. Annu Rev Mar Sci 4: 467–493

Christou ED, Zervoudaki S, Fernandez De Puelles ML, Protopapa M and others (2017) Response of the calanoid copepod *Clausocalanus furcatus*, to atmospheric deposition events: outcomes from a mesocosm study. Front Mar Sci 4:35

Cornell S, Randell A, Jickells T (1995) Atmospheric inputs of dissolved organic nitrogen to the oceans. Nature 376: 243–246

Countway PD, Gast RJ, Savai P, Caron DA (2005) Protistan diversity estimates based on 18S rDNA from seawater incubations in the western North Atlantic. J Eukaryot Microbiol 52:95–106

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. Nat Methods 9:772

Díez B, Pedros-Alio C, Marsh TL, Massana R (2001) Application of denaturing gradient gel electrophoresis (DGGE) to study the diversity of marine picoeukaryotic assemblages and comparison of DGGE with other molecular techniques. Appl Environ Microbiol 67:2942–2951

Duce RA, LaRoche J, Altieri K, Arrigo KR and others (2008) Impacts of atmospheric anthropogenic nitrogen on the open ocean. Science 320:893–897

Gallisai R, Peters F, Volpe G, Basart S, Baldasano JM (2014) Saharan dust deposition may affect phytoplankton growth in the Mediterranean Sea at ecological time scales. PLOS ONE 9:e110762

Gallisai R, Volpe G, Peters F (2016) Large Saharan dust storms: implications for chlorophyll dynamics in the Mediterranean Sea. Global Biogeochem Cycles 30: 1725–1737

Giner CR, Forn I, Romac S, Logares R and others (2016) Environmental sequencing provides reasonable estimates of the relative abundance of specific picoeukaryotes. Appl Environ Microbiol 82:4757–4766

Guerzoni S, Chester R, Dulac F, Herut B and others (1999) The role of atmospheric deposition in the biogeochemistry of the Mediterranean Sea. Prog Oceanogr 44: 147–190

Guillou L, Bachar D, Audic S, Bass D and others (2013) The Protist Ribosomal Reference database (PR^2): a catalog of unicellular eukaryote small sub-unit rRNA sequences with curated taxonomy. Nucleic Acids Res 41:D597–D604

Guindon S, Dufayard JF, Lefort V, Anisimova M and others (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst Biol 59:307–321

Guo C, Yu J, Ho TY, Wang L and others (2012) Dynamics of phytoplankton community structure in the South China Sea in response to the East Asian aerosol input. Biogeosciences 9:1519–1536

Guo C, Xia X, Pitta P, Herut B and others (2016) Shifts in microbial community structure and activity in the ultraoligotrophic eastern Mediterranean Sea driven by the deposition of Saharan dust and European aerosols. Front Mar Sci 3:170

Haas BJ, Gevers D, Earl AM, Feldgarden M and others (2011) Chimeric 16S rRNA sequence formation and detection in Sanger and 454-pyrosequenced PCR amplicons. Genome Res 21:494–504

Hagino K, Onuma R, Kawachi M, Horiguchi T (2013) Discovery of an endosymbiotic nitrogen-fixing cyanobacterium UCYN-A in *Braarudosphaera bigelowii* (Prymnesiophyceae). PLOS ONE 8:e81749

Herut B, Krom MD, Pan G, Mortimer R (1999) Atmospheric input of nitrogen and phosphorus to the Southeast Mediterranean: sources, fluxes, and possible impact. Limnol Oceanogr 44:1683–1692

Herut B, Collier R, Krom MD (2002) The role of dust in supplying nitrogen and phosphorus to the Southeast Mediterranean. Limnol Oceanogr 47:870–878

Herut B, Rahav E, Tsagaraki TM, Giannakourou A and others (2016) The potential impact of Saharan dust and polluted aerosols on microbial populations in the East Mediterranean Sea, an overview of a mesocosm experimental approach. Front Mar Sci 3:226

Holm-Hansen O, Lorenzen CJ, Holmes RW, Strickland JDH (1965) Fluorometric determination of chlorophyll. ICES J Mar Sci 30:3–15

Ito T, Nenes A, Johnson MS, Meskhidze N, Deutsch C (2016) Acceleration of oxygen decline in the tropical Pacific over the past decades by aerosol pollutants. Nat Geosci 9:443–447

Ivančič I, Degobbis D (1984) An optimal manual procedure for ammonia analysis in natural waters by the indophenol blue method. Water Res 18:1143–1147

Jones SE, Lennon JT (2010) Dormancy contributes to the maintenance of microbial diversity. Proc Natl Acad Sci USA 107:5881–5886

Kanakidou M, Duce RA, Prospero JM, Baker AR and others (2012) Atmospheric fluxes of organic N and P to the global ocean. Global Biogeochem Cycles 26:GB3026

Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol 30:772–780

Kim DY, Countway PD, Gast RJ, Caron DA (2011) Rapid shifts in the structure and composition of a protistan assemblage during bottle incubations affect estimates of total protistan species richness. Microb Ecol 62:383–398

Koid A, Nelson WC, Mraz A, Heidelberg KB (2012) Comparative analysis of eukaryotic marine microbial assemblages from 18S rRNA gene and gene transcript clone libraries by using different methods of extraction. Appl Environ Microbiol 78:3958–3965

Krom MD, Kress N, Brenner S, Gordon LI (1991) Phosphorus limitation of primary productivity in the eastern Mediterranean Sea. Limnol Oceanogr 36:424–432

Krom MD, Herut B, Mantoura RFC (2004) Nutrient budget for the eastern Mediterranean: implications for phosphorus limitation. Limnol Oceanogr 49:1582–1592

Le Moal M, Collin H, Biegala IC (2011) Intriguing diversity among diazotrophic picoplankton along a Mediterranean transect: a dominance of rhizobia. Biogeosciences 8: 827–840

Lekunberri I, Lefort T, Romero E, Vázquez-Domínguez E and others (2010) Effects of a dust deposition event on coastal marine microbial abundance and activity, bacterial community structure and ecosystem function. J Plankton Res 32:381–396

Liu H, Probert I, Uitz J, Claustre H and others (2009) Extreme diversity in noncalcifying haptophytes explains a major pigment paradox in open oceans. Proc Natl Acad Sci USA 106:12803–12808

Logares R, Audic S, Bass D, Bittner L and others (2014) Patterns of rare and abundant marine microbial eukaryotes. Curr Biol 24:813–821

López-García P, Philippe H, Gail F, Moreira D (2003) Autochthonous eukaryotic diversity in hydrothermal sediment and experimental microcolonizers at the Mid-Atlantic Ridge. Proc Natl Acad Sci USA 100:697–702

Loÿe-Pilot MD, Martin JM, Morelli J (1986) Influence of Saharan dust on the rain acidity and atmospheric input to the Mediterranean. Nature 321:427–428

Man-Aharonovich D, Philosof A, Kirkup BC, Le Gall F and others (2010) Diversity of active marine picoeukaryotes in the eastern Mediterranean Sea unveiled using photosystem-II *psb*A transcripts. ISME J 4:1044–1052

Marie D, Brussaard CPD, Thyrhaug R, Bratbak G, Vaulot D (1999) Enumeration of marine viruses in culture and natural samples by flow cytometry. Appl Environ Microbiol 65:45–52

Marín I, Nunes S, Sánchez-Pérez ED, Aparicio FL and others (2017a) Anthropogenic versus mineral aerosols in the stimulation of microbial planktonic communities in coastal waters of the northwestern Mediterranean Sea. Sci Total Environ 574:553–568

Marín I, Nunes S, Sánchez-Pérez ED, Txurruka E and others (2017b) Coastal bacterioplankton metabolism is stimulated stronger by anthropogenic aerosols than Saharan dust. Front Microbiol 8:2215

Marquardt M, Vader A, Stübner EI, Reigstad M, Gabrielsen TM (2016) Strong seasonality of marine microbial eukaryotes in a high-Arctic fjord (Isfjorden, in West Spitsbergen, Norway). Appl Environ Microbiol 82:1868–1880

Massana R (2011) Eukaryotic picoplankton in surface oceans. Annu Rev Microbiol 65:91–110

Massana R, Guillou L, Terrado R, Forn I, Pedrós-Alió C (2006) Growth of uncultured heterotrophic flagellates in unamended seawater incubations. Aquat Microb Ecol 45:171–180

Massana R, Gobet A, Audic S, Bass D and others (2015) Marine protist diversity in European coastal waters and sediments as revealed by high-throughput sequencing. Environ Microbiol 17:4035–4049

Meador TB, Goldenstein NI, Gogou A, Herut B and others (2017) Planktonic lipidome responses to aeolian dust input in low-biomass oligotrophic marine mesocosms. Front Mar Sci 4:113

Mercier C, Boyer F, Bonin A, Coissac E (2013) SUMATRA and SUMACLUST: fast and exact comparison and clustering of sequences. Available at http://metabarcoding.org/sumatra

Mills MM, Ridame C, Davey M, La Roche J, Geider RJ (2004) Iron and phosphorus co-limit nitrogen fixation in the eastern tropical North Atlantic. Nature 429:292–294

Oksanen J, Blanchet FG, Kindt R, Legendre P and others (2014) vegan: community ecology package. R package version 2.2-0. Available at http://CRAN.R-project.org/package=vegan/

Pedrós-Alió C (2012) The rare bacterial biosphere. Annu Rev Mar Sci 4:449–466

Peloquin J, Swan C, Gruber N, Vogt M and others (2013) The MAREDAT global database of high performance liquid chromatography marine pigment measurements. Earth Syst Sci Data 5:109–123

Pulido-Villena E, Wagener T, Guieu C (2008) Bacterial response to dust pulses in the western Mediterranean: Implications for carbon cycling in the oligotropic ocean. Glob Biogeochem Cycles 22:GB1020

R Core Team (2014) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. Available at www.R-project.org

Rahav E, Shun-Yan C, Cui G, Liu H and others (2016) Evaluating the impact of atmospheric depositions on springtime dinitrogen fixation in the Cretan Sea (eastern Mediterranean) — a mesocosm approach. Front Mar Sci 3:180

Rimmelin P, Moutin T (2005) Re-examination of the MAGIC method to determine low orthophosphate concentration in seawater. Anal Chim Acta 548:174–182

Sciare J, Bardouki H, Moulin C, Mihalopoulos N (2003) Aerosol sources and their contribution to the chemical composition of aerosols in the eastern Mediterranean Sea during summertime. Atmos Chem Phys 3:291–302

Shi XL, Marie D, Jardillier L, Scanlan DJ, Vaulot D (2009) Groups without cultured representatives dominate eukaryotic picophytoplankton in the oligotrophic South East Pacific Ocean. PLOS ONE 4:e7657

Shi XL, Lepère C, Scanlan DJ, Vaulot D (2011) Plastid 16S rRNA gene diversity among eukaryotic picophytoplankton sorted by flow cytometry from the South Pacific Ocean. PLOS ONE 6:e18979

Siokou-Frangou I, Christaki U, Mazzocchi MG, Montresor M and others (2010) Plankton in the open Mediterranean Sea: a review. Biogeosciences 7:1543–1586

Steemann Nielsen E (1965) On the determination of the activity in ^{14}C-ampoules for measuring primary production. Limnol Oceanogr 10:R247–R252

Stoeck T, Zuendorf A, Breiner HW, Behnke A (2007) A molecular approach to identify active microbes in environmental eukaryote clone libraries. Microb Ecol 53: 328–339

Strickland JDH, Parsons TR (1972) A practical handbook of seawater analysis, 2nd edn. Fisheries Research Board of Canada, Ottawa

Thompson AW, Foster RA, Krupke A, Carter BJ and others (2012) Unicellular cyanobacterium symbiotic with a single-celled eukaryotic alga. Science 337:1546–1550

Tsagaraki TM, Herut B, Rahav E, Berman Frank IR and others (2017) Atmospheric deposition effects on plankton communities in the eastern Mediterranean: a mesocosm experimental approach. Front Mar Sci 4:210

Tsiola A, Tsagaraki TM, Giannakourou A, Nikolioudakis N and others (2017) Bacterial growth and mortality after deposition of Saharan dust and mixed aerosols in the eastern Mediterranean Sea: a mesocosm experiment. Front Mar Sci 3:281

Unrein F, Gasol JM, Not F, Forn I, Massana R (2014) Mixotrophic haptophytes are key bacterial grazers in oligotrophic coastal waters. ISME J 8:164–176

Wang SH, Hsu NC, Tsay SC, Lin NH and others (2012) Can Asian dust trigger phytoplankton blooms in the oligotrophic northern South China Sea? Geophys Res Lett 39: L05811

Westrich JR, Ebling AM, Landing WM, Joyner JL and others (2016) Saharan dust nutrients promote *Vibrio* bloom formation in marine surface waters. Proc Natl Acad Sci USA 113:5964–5969

Zubkov MV, Tarran GA (2008) High bacterivory by the smallest phytoplankton in the North Atlantic Ocean. Nature 455:224–226

Editorial responsibility: Klaus Jürgens, Rostock, Germany

Submitted: October 26, 2017; Accepted: June 25, 2018
Proofs received from author(s): August 28, 2018