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UNIVERSITY OF CALIFORNIA
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BIOAEROSOLS: ABUNDANCE, DIVERSITY, AND IMPACTS ON MARINE SYSTEMS

A dissertation submitted in partial satisfaction
of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

EARTH SCIENCES

By

Esra Mescioglu

June 2020

The Dissertation of Esra Mescioglu
is approved:

____
Professor Matthew Clapham

____
Dr. Adina Paytan

____
Dr. Marilou Sison-Mangus

____
Quentin Williams
Acting Vice Provost and Dean of Graduate Studies

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ABSTRACT

Bioaerosol: Abundance, Diversity, and Impacts on Marine Systems

Esra Mescioglu

Atmospheric aerosols (suspension of solid or liquid particles in the present in the atmosphere) can harbor a diverse array of airborne microorganisms and upon deposition into terrestrial or marine systems, airborne microbes can alter function and biogeochemical cycles of the receiving ecosystems. My work focuses on aerosols and their impact on marine environments. The three chapters in this thesis examine 1) the abundance and diversity of airborne microorganisms over the Mediterranean Sea, 2) the impacts of airborne microorganisms on the northern Red Sea surface water microbial diversity and function, and 3) the efficiency of three types of aerosol sampling instrumentation. In the first study, I described the abundance and genetic diversity of airborne bacteria in air samples collected over an East-West transect of the entire Mediterranean Sea and investigated 1) the controls on the diversity of airborne microbes, and 2) the source of the microbes comprising the aerosol microbiome over the Mediterranean Sea. The results show that airborne bacteria represent diverse groups with the most abundant bacteria from the Firmicutes (Bacilli and Clostridia) and Proteobacteria (Alphaproteobacteria, Betaproteobacteria, and Gammaproteobacteria) phyla. Most of the bacteria in the samples have previously been observed in the air at other open ocean locations, in the air over the Mediterranean Sea during dust storms, and in surface water of the Mediterranean Sea. The results demonstrate that airborne

bacterial diversity is positively correlated with the mineral dust content in the aerosols and the taxonomic composition differed between major basins of the Mediterranean Sea. In the second study, a mesocosm experiment was conducted to elucidate 1) how bioaerosols collected during dust-events impact prokaryotic and eukaryotic relative abundance in the northern Red Sea (NRS) surface water, and 2) how the changes in community structure affect biogeochemical cycles of the NRS. Results show that the airborne microorganisms and viruses suppressed primary production (as much as 50%), increased bacterial production (as much as 55%), and decreased the diversity of eukaryotes. These results suggest that airborne microorganisms have implications for the carbon cycle in low nutrient low chlorophyll marine ecosystems. The final chapter tests the efficiency of samples collected with three instruments (a membrane filtration device, a liquid impinger, and a portable electrostatic precipitator bioaerosol collector) for culture-dependent (colony-forming units) and culture-independent (DNA yield) studies. The results show that the electrostatic precipitator collected microorganisms significantly more efficiently than the membrane filtration and liquid impingement in both types of studies over the same time interval, primarily due to its higher flowrate.

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INTRODUCTION

Atmospheric deposition is an important source of micro- and macro-nutrients, metals, and various pollutants into aquatic systems, and it is widely accepted that these inputs can alter aquatic ecosystems and biogeochemical cycles in the ocean (Guieu et al., 2014). The transport and deposition of terrestrial dust into the oceans is a major source of iron (Fe) (Duce et al., 1991) and is especially important in high nutrient low chlorophyll (HNLC) Fe-limited ocean regions. Fe inputs have been shown to have a fertilization effect on phytoplankton in these HNLC areas (Falkowski et al., 1998), impacting primary productivity and, therefore, the carbon cycle. Like Fe, addition of atmospheric phosphorus (P) has been shown to impact phytoplankton populations in P-limited waters (Mackey et al., 2012; Chien et al., 2016). Atmospheric input of Fe and P can also stimulate N_2 fixation in the ocean (Mills et al., 2004; Falkowski et al., 1998; Gruber and Sarmiento, 1997; Michaels et al., 1996). On the other hand, atmospheric deposition of toxic metals and pollutants can cause declines in populations of some algal species (Paytan et al., 2009; Krom et al., 2016; Rahav et al., 2020). The central topic of my dissertation is bioaerosols in the marine environment. This is a distinct component of the field of environmental aeromicrobiology, the study of microorganisms in the atmosphere, which has not been as thoroughly studied in marine settings. My work sheds light on the abundance, diversity, and impacts of bioaerosols on marine systems. Bioaerosols are defined as airborne organisms and particles of biological origin and can be used to refer to a range of airborne biological matter. In

this thesis the term will be used to refer specifically to airborne prokaryotes (bacteria and archaea) and eukaryotes (fungi, fungal spores and algae).

Abundance of Microbes in Aerosols

Biological matter originating from the terrestrial and marine environment can be lifted as aerosols in the atmosphere by wind and wave action, respectively, and can travel over vast distances. Mayol et al. (2017) measured the abundance of prokaryotes and eukaryotes during the Malaspina 2010 Circumnavigation Expedition that sailed the tropical and subtropical Atlantic, Indian and Pacific Oceans. They found that abundances of prokaryotes in aerosol collected using a cyclonic aerosol collector were variable, ranging from 5×10^2 and 8×10^4 cells m^{-3} air (median 6.7×10^3 cells m^{-3} air) and unicellular eukaryote concentrations in the air above the ocean ranged from 1×10^2 and 1.8×10^4 cells m^{-3} air (median 3.2×10^3 cells m^{-3} air). They reported that the median values recorded were comparable to microbial abundances over the ocean derived from global bioaerosol emission and transport models (Spracklen & Heald, 2014; Burrows et al., 2009). These numbers are relatively low compared to the typical number of microorganisms in surface seawater (5×10^5 prokaryotic cells mL^{-1} seawater) (Whitman et al., 1999). Direct counts from samples collected in coastal marine sites indicate that prokaryotic abundances are closer to the high end of the range recorded by Mayol et al., [2017] (8.0×10^4 cells m^{-3} air) (Cho & Hwang, 2011) while the concentrations of eukaryotes are similar in coastal and open ocean sites (DeLeon-Rodriguez et al., 2013).

Data collected during high atmospheric deposition events, such as dust storms, generally show much higher abundance of both prokaryotes and eukaryotes, reaching concentrations an order of magnitude greater than during non-dust events (Griffin et al., 2001). This suggests a relation between the aerosol concentration in the air and bioaerosol abundance in the same airmass. Indeed, a study at the Turkish Mediterranean coastline reported a significant correlation between dust deposition and prevalence of culturable microorganisms from aerosol samples (Griffin et al. 2007) and a similar trend was observed over the mid-Atlantic Ocean (Griffin et al., 2006). These results are consistent with studies that show that airborne bacteria are mostly associated with particles in the air, including mineral dust (Polymenakou et al., 2008; Yamaguchi et al., 2012; Després et al., 2012.

Due to this spatial variation in microbial abundance, more research focusing on site specific data as well as comprehensive long-term monitoring would benefit the field.

Microbial Diversity in Aerosols over the Ocean

The diversity of microbes in outdoor air has been shown to vary between environments (Kaarakainen et al., 2008), yet there are only a few studies investigating airborne microbial diversity over the ocean. Despite the limited number of studies a large diversity of microorganisms has been reported in aerosol samples collected above the ocean (Table 1). The identified organisms originate from both terrestrial and marine sources, and include a variety of human, animal, and plant pathogens, as well as

microorganisms known to be important in marine systems, such as cyanobacteria (Table 1). The existing information we have on airborne microorganisms over the ocean focuses on bacteria and fungi, and has been generated using traditional culturing methods as well as 16S rRNA sequencing techniques (bacteria). The few studies conducted so far indicate that airborne microbes are found ubiquitously over marine environments, however the communities are not homogenous (Xia et al., 2011) and the specific compositions and factors driving the diversity at different locations of time are still poorly constrained (Griffin et al., 2007; Guieu et al., 2014). In general, there is less information about airborne eukaryotes over marine systems.

Origin of airmass and atmospheric conditions have been identified as drivers of bioaerosol diversity by several of the studies [Griffin et al., 2001; Rahav et al., 2016a; Mazar et al., 2016; Gat et al., 2017]. Origin of airmass refers to the path that the airmass followed before arriving at the sampling site, whereas the condition of airmass refers to humidity, cloud-cover, acidity of the air or whether there was an atmospheric event such as a dust storm or rain at the time of sampling. These two factors are linked, because the origin of the airmass can be influenced by atmospheric conditions and vice versa (reviewed in Griffin et al., 2002). Specifically, during dust events the origin of airmass is likely an arid landmass, and this leads to relatively more soil-associated organisms (Mazar et al., 2016; Maki et al., 2014). However, since topsoil microbial communities differ between different arid environments (Gat et al., 2017; Bahram et al., 2018), bioaerosol diversity and community composition is not homogenous during

dust events that originate from different locations (Rahav et al., 2016a; Gat et al., 2017), and it is important to determine the origin of landmass from which the mineral dust was uplifted for each bioaerosol sample.

Similar to the differences in microbial abundance during dust storms, the microbial diversity in aerosols is higher during dust events than under background non-dust event conditions. Griffin et al. (2003) tested the diversity of bioaerosols at a coastal sampling site in the U.S. Virgin Islands and reported 5 unique species of bacteria (all gram positives) and 3 unique species of fungi during non-dust events, while during an African dust event they found 42 unique species of bacteria (gram positive and gram negative) and 7 unique species of fungi. They also reported more pathogens in the samples collected during dust events (Griffin et al., 2003). Smith et al. (2012) collected aerosols at the Mt. Bachelor Observatory before, during, and after an Asian long-range transport dust event, and found that the bacterial richness was higher during dust events in comparison to before and after dust events (background conditions). Mazar et al. (2016) collected aerosol samples in the Mediterranean coastal city of Rehovot, Israel during dust events and a non-dust events, and reported significantly higher number of observed operational taxonomic units (OTUs), which signify the number of unique microorganisms, during dust events. The community composition during dust events also had a higher proportion of bacteria from local sand desert soils (Mazar et al., 2016). Gat et al. (2017) later reported that dust storms arriving in Rehovot, Israel, from different origins exhibited distinct bacterial communities.

Most of the studies looking at bioaerosol diversity in marine settings were conducted in coastal regions with far fewer studies in the open ocean. Xia et al. (2015) collected aerosol samples over remote marine regions, including the western Pacific Ocean, northern Pacific Ocean, the Antarctic Ocean, and the Norwegian Sea and their results showed differences in diversity among regions. They suggested that the source of airmasses influenced the microbial community compositions in the aerosols. One of the significant sources of aerosol particles in the air over the open ocean is the surface layer of the ocean from which marine microbes can become aerosolized through movements of water and wind, leading to sea spray and bubble-bursting (Rastelli et al., 2017). Cho and Hwang [2011] used 16S rRNA gene sequencing to identify airborne bacteria over the East China Sea during normal atmospheric conditions (non-dust events) and found that the community was composed of both terrestrial and marine organisms. Mayol et al. (2017) also found that a substantial percentage of airborne microbes over the ocean (33-68%) had marine origin.

Table I.1.

Class	Order	Family	Genus	Species	Information as given by Author	Study
-	-	-	Unidentified	proteobacterium	Grouped with Zoogloea sp., Marine isolate	3
-	-	-	Unidentified		Rape rhizosphere isolate. 98% homology (656/654) with Curtobacterium sp.	3
-	-	-	Unidentified		Causes red stripes in rice and is 98% homologous (685/694) to M. testaceum	3
-	-	-	Unidentified		Trickling filter isolate. 97% homology (641/659) to Sphingomonas sp. (marine isolate)	3
-	-	-	Unknown		Arizona soil isolate	3
Acidimicrobiia	-	-	-	-		1
Actinobacteria	-	-	-	-		1
Actinobacteria	Actinomycetales	Microbacteriaceae	Microbacterium	imperiale	Species of this genus commonly isolated in environmental studies	2
Actinobacteria	Actinomycetales	Nocardiaceae	Rhodococcus	-	human pathogen	5
Actinobacteria	Propionibacteriales	Propionibacteriaceae	Propionibacterium	-	Id'd same to many species in this genus, 3 of 7 matches were P. acnes a known human pathogen	2
Actinobacteria	Propionibacteriales	Propionibacteriaceae	Propionibacterium	acnes	human pathogen	5
Actinomycetes	Actinomycetales	Microbacteriaceae	Curtobacterium	albidum	Rice isolate	3
Actinomycetes	Actinomycetales	Microbacteriaceae	Curtobacterium	citreum	Bean pathogen	3

Actinomycetes	Actinomycetales	Microbacteriaceae	Curtobacterium	citreum	Bean pathogens	3
Actinomycetes	Actinomycetales	Microbacteriaceae	Curtobacterium	citreum	Bean pathogens	3
Actinomycetes	Actinomycetales	Microbacteriaceae	Curtobacterium	luteum	Soil Bacteria	3
Actinomycetes	Actinomycetales	Microbacteriaceae	Curtobacterium	luteum	Soil bacteria	3
Actinomycetes	Actinomycetales	Microbacteriaceae	Microbacterium	arborescens	Soil bacteria	3
Actinomycetes	Actinomycetales	Microbacteriaceae	Microbacterium	sp.	Soil bacteria	3
Actinomycetes	Actinomycetales	Microbacteriaceae	Microbacterium	testaceum	Soil bacteria	3
Actinomycetes	Actinomycetales	Micrococcaceae	Arthrobacter	globiformis	Common non-pathogenic environmental	3
Actinomycetes	Actinomycetales	Micrococcaceae	Arthrobacter	sp.	PCB degrading soil bacteria and human	3
Actinomycetes	Actinomycetales	Micrococcaceae	Kocuria	erythromyxa	Marine isolate	3
Actinomycetes	Actinomycetales	Streptomycetaceae	Streptomyces	-	Laboratory contaminate. Id'd equally to S. thermotolerans and S. bellus	2
Bacteroidetes	-	-	-	-		4
Bacteroidetes	Bacteroidales	Dysgonamonadaceae	Dysgonomonas	gadei strain		4
Bacteroidetes	Flavobacteriales	Cytophagaceae	Leeuwenhoekiella	aequorea LMG		4
Bacteroidetes	Flavobacteriales	Flavobacteriaceae	Bergeyella	sp. M1T8B8		4
Flavobacteriia	-	-	-	-		1
-	-	-	-	-		1
Cytophagia	-	-	-	-		1
Deferribacteres	-	-	-	-		1
Thermoplasmata	-	-	-	-		1
Bacilli	-	-	-	-		1

Bacilli	Bacillales	Bacillaceae	-	-		5
Bacilli	Bacillales	Bacillaceae	-	-	Match at 99% to Bacillus barbaricus (628/633)	2
Bacilli	Bacillales	Bacillaceae	Bacillus	firmus		4
Bacilli	Bacillales	Bacillaceae	Bacillus	megaterium	Tree pathogen	2
Bacilli	Bacillales	Bacillaceae	Bacillus	megaterium	Elm pathogen	3
Bacilli	Bacillales	Bacillaceae	Bacillus	megaterium	Elm pathogen	3
Bacilli	Bacillales	Bacillaceae	Bacillus	pumilus	Potential human pathogen and common environmental isolate	2
Bacilli	Bacillales	Bacillaceae	Bacillus	pumilus	Most frequently recovered bacilli in Ethiopian spices	3
Bacilli	Bacillales	Bacillaceae	Bacillus	thuringiensis	Insect pathogen. Used as a mosquito control agent. Common soil isolates	2
Bacilli	Bacillales	Bacillaceae	Lysinibacillus	boronitolerans		5
Bacilli	Bacillales	Paenibacillaceae	Brevibacillus	reuszeri		5
Bacilli	Bacillales	Planococcaceae	-	-		5
Bacilli	Bacillales	Staphylococcaceae	Staphylococcus	-	human pathogen	5
Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	infantis	human pathogen	5
Alphaproteobacteria	-	-	-	-		1
Alphaproteobacteria	Bradyrhizobiaceae	Bradyrhizobiaceae	Afipia	genosp. 11	Human isolate/pathogen	2
Alphaproteobacteria	Caulobacterales	Caulobacteraceae	-	-		5
Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Brevendimonas	-		5
Alphaproteobacteria	Rhizobiales	Brucellaceae	-	-	marine bacteria	5
Alphaproteobacteria	Rhizobiales	Brucellaceae	Ochrobactrum	-		5

Alphaproteobacteria	Rhizobiales	Methylobacteriaceae	Methylobacterium	adhaesivum		5
Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Sinorhizobium	sp.	Plant symbiont	3
Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Paracoccus	aminivorans	Activated sludge isolate. Thiocyanate-utilizing facultative chemolithotroph	3
Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Paracoccus	sp.	Marine isolate	3
Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Ruegeria	sp. PR1b		4
Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	Gluconacetobacter	-		5
Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	-	-	marine bacteria	5
Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	-	Id'd to S. pruni and S. aquatilis. Common environmental isolate. S. pruni is a plant pathogen (information from GenBank match submission data)	2
Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	-		5
Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	pruni	Plant pathogen	3
Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	sp.	Plant/marine/soil isolate	3
Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	trueperi	Soil isolate	3
Betaproteobacteria	-	-	-	-		1
Betaproteobacteria	-	-	-	-		4
Betaproteobacteria	Burkholderiales	Alcaligenaceae	-	-		5
Betaproteobacteria	Burkholderiales	Alcaligenaceae	Achromobacter	sp. JDM-3-04		4
Betaproteobacteria	Burkholderiales	Burkholderiaceae	Cupriavidus	-		5
Betaproteobacteria	Burkholderiales	Comamonadaceae	Delftia	-		4
Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Duganella	-	Beta Proteobacteria. Genus DNA has been	2

					identified in human disease studies	
Deltaproteobacteria	-	-	-	-		1
Epsilonproteobacteria	Campylobacterales	Campylobacteraceae	Arcobacter	cryaerophilus		4
Gammaproteobacteria	-	-	-	-		1
Gammaproteobacteria	Alteromonadales	Alteromonadaceae	Alteromonas	macleodii strain		4
Gammaproteobacteria	Alteromonadales	Alteromonadaceae	Alteromonas	sp. NBF18		4
Gammaproteobacteria	Alteromonadales	Idiomarinaceae	Idiomarina	sp. JL1018		4
Gammaproteobacteria	Alteromonadales	Pseudoalteromonadaceae	Pseudoalteromonas	-	marine bacteria	5
Gammaproteobacteria	Alteromonadales	Pseudoalteromonadaceae	Pseudoalteromonas	sp.		4
Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	-	-	human pathogen	5
Gammaproteobacteria	Oceanospirillales	Halomonadaceae	-	-		5
Gammaproteobacteria	Oceanospirillales	Halomonadaceae	Halomonas	aquamarina strain	marine	4
Gammaproteobacteria	Pseudomonadales	Moraxellaceae	-	-		5
Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	-	Common environmental soil and water isolates. Id'd equally to A. johnsonii, junii, anitratus, etc.	2
Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	-	-		5
Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	-	Common environmental soil and water isolates (information from GenBank match submission data)	2
Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	-		5
Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	alcalophila	Marine isolate	3
Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	oleovorans	Cooking oil waste isolate	3

Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	riboflavina	Soil isolate	3
Gammaproteobacteria	Xanthomonadales	Rhodanobacteraceae	Rhodanobacter	terrae		5

Table I.1. Bacteria identified over marine systems by previous studies. The numbers in the study column refer to which study reported each organism (1=Mayol et al., 2017, 2=Griffin et al., 2003, 3=Griffin et al., 2006, 4=Cho & Hwang, 2011, and 5=Xia et al., 2015).

Table I.2.

Class	Order	Family	Genus	Species	Information as given by Author	Study
Dothideomycetes	Capnodiales	Cladosporiaceae	Cladosporium	-	Common environmental isolate, plant and human pathogenic species	1
Dothideomycetes	Capnodiales	Cladosporiaceae	Cladosporium	cladosporioides	Common environmental isolate. Human and plant pathogen	2
Dothideomycetes	Capnodiales	Davidiellaceae	Cladosporium	-	Common environmental isolates, plant and human pathogenic species	1
Dothideomycetes	Dothideales	Dothioraceae	Aureobasidium	-	Common temperate-climate plant leaf isolates. Can cause skin infections. Rare cases of systemic infection in immunocompromised patients	1
Dothideomycetes	Pleosporales	Pleosporaceae	Cochliobolus	sativus	Grass pathogen	2
Dothideomycetes	Pleosporales	Pleosporaceae	Pleospora	rudis	Plant isolate	2
Eurotiomycetes	Eurotiales	Trichocomaceae	Penicillium	-	Common temperate-climate isolate. P. marneffie can cause lymphatic infections	1
Eurotiomycetes	Onygenales	Arthrodermataceae	Microsporium	-	5 human and 7 animal pathogens of 17 known species. Skin and hair infections	1
Eurotiomycetes	Onygenales	Arthrodermataceae	Microsporium	-	5 human and 7 animal pathogens of 17 known species. Skin and hair infections	1
Eurotiomycetes	Onygenales	Arthrodermataceae	Trichophyton	-	11 human and 4 animal hair, scalp, and skin pathogens of 22 known species	1
Eurotiomycetes	Onygenales	Arthrodermataceae	Trichophyton	-	11 human and 4 animal hair, scalp, and skin pathogens of 22 known species	1
Eurotiomycetes	Onygenales	Onygenaceae	Chrysosporium	-	Common plant and soil isolate. Rare cases of human pathogenicity	1
Lecanoromycetes	incertae sedis	Coccodiniaceae	Coccodinium	bartschii	aka – sooty mold	2
Leotiomycetes	Helotiales	incertae sedis	Scytalidium	-	Common tropical plant and soil isolate	1

Onygenales	Onygenales	Arthrodermataceae	Trichophyton	-	11 human and 4 animal hair, scalp, and skin pathogens of 22 known species	1
Sordariomycetes	Hypocreales	Nectriaceae	Gibberella	pulicaris	Causes potato dry rot (storage disease)	2
Zygomycetes	Mucorales	Mucoraceae	Rhizomucor	-	Common environmental isolate. R. pusillus can cause infections in the immunocompromised	1

Table I.2. Fungi identified over marine systems by previous studies. The numbers in the study column refer to which study reported each organism (1=Griffin et al., 2003, 2=Griffin et al., 2006)

Impacts of Bioaerosols Deposited into the Surface Ocean

After deposition, as much as 20% of bioaerosols can remain viable (Pósfai et al., 2003; Prospero et al., 2005; Gorbushina et al., 2007; Deguillaume et al., 2008; Womack et al., 2010; Polymenakou, 2012; Mayol, 2017), and can impact ambient communities in aquatic systems. For example, bioaerosols have been shown to cause declines in populations of marine macro-organisms such as corals. Shinn et al. (2000) proposed that *Aspergillus sydowii*, a terrestrial fungus, was lifted and transported into the Caribbean Sea waters by dust storms originating in Africa and was the cause of white-band disease in corals. Garrison et al. (2003) later supported this hypothesis and added that recent changes in the amount and composition of dust have contributed to noticeable increases in coral disease in the Caribbean. Weir et al. (2004) tested and provided evidence for this hypothesis by isolating *Aspergillus spp.* from African dust collected in the Caribbean, inoculating gorgonian coral with the fungi isolated, and re-isolating the original pathogen from the coral.

More recently, studies have demonstrated that viable airborne microbes may contribute to geochemical processes in the surface water. Rahav et al., [2016a] conducted a bioassay experiment where bioaerosols associated with three types of dust were incubated in sterile Mediterranean Sea water, showing an increase of bacterial production and N_2 fixation. Their results suggested that bioaerosols could account for up to 50% of bacterial production and a substantial fraction of N_2 fixation in the Mediterranean immediately following dust events. In another study, Rahav et al.

(2016b) compared production rates of bioaerosols added to Mediterranean Sea surface water (sterile and non-sterile) and found that bioaerosols comprised 30-50% of heterotrophs and accounted for a significant fraction (13-42%) of bacteria production when adding aerosol amounts comparable to those deposited during dust storms. The airborne microbes, through carbon and N_2 fixation, can therefore impact nutrient cycles. Finally, airborne microbes and viruses can also interact with marine bacteria and phytoplankton and lead to a change in microbial ecology as indicated by the changes in community structure and production rates (Rahav et al., 2016a). Sharoni et al. (2015) reported on aerosolization of EhV, a lytic large doublestranded DNA coccolithovirus that infects *Emiliania huxleyi,* a bloom-forming phytoplankton, and the dispersal of these viruses with sea-spray. They hypothesized that this phenomenon is an effective transmission mechanism for spreading viral infection, and can impact host-virus dynamics.

Future Needs for Marine Aeromicrobiology

Although the importance of studying airborne microbes is well documented in indoor setting (particularly in areas like hospitals) and when related to agriculture or human disease transmission (like the fungal disease esca that devastates vines or the flu), the field of aeromicrobiology in natural systems, and especially marine systems, is still in its infancy (Smith, 2013). The environmental aeromicrobiology data we do have is from sporadic projects as opposed to more systematic surveys and investigations. To

date, the diversity and ecological impacts of bioaerosols upon deposition into seawater remain sparsely documented and poorly understood.

Currently, little is known about how bioaerosols affect native phytoplankton and bacterial populations. The impact may be particularly important in low-nutrient, low-chlorophyll (LNLC) marine ecosystems where the surface ocean biomass is low (Guieu et al., 2014). LNLC marine environments make up 60% of the global oceans (Antoine et al., 1996) and are expanding (15% increase from 1998 to 2006) (Polovina et al., 2008). Although atmospheric deposition is an especially important source of nutrients and trace metals in LNLC systems (Jickells et al., 2005; Duce et al., 2008; Herut et al., 1999, 2002; Krom et al., 2004), impacts of bioaerosols on LNLC regions remain understudied. Studies that improve our understanding of how these geographically vast areas of ocean will be impacted by climate change and contribute to the global carbon cycle will be of great value as existing data do not provide a clear prediction. For my dissertation, I have focused on two ecologically distinct LNLC systems that are frequently exposed to high atmospheric aerosol deposition: the Mediterranean Sea (MS) and the Northern Red Sea (NRS), and investigated abundance, diversity and impacts on marine microbial ecology.

Methods used in the Study of Environmental Aeromicrobiology

Environmental aeromicrobiology studies have utilized different sample collection protocols, devices, and analytical assays (Gandolfi et al. 2013, Behzad et al. 2015).

While the two most common instrumentation used to collect bioaerosols are membrane filtration devices and liquid impingement devices (Fahlgren et al. 2011; Fields et al. 1974; Jensen et al. 1992; Kesavan et al. 2010; Griffin et al. 2001; Buttner et al. 1997), there are various options available including cyclonic collectors (Mayol et al., 2017) and electrostatic precipitators (Mbareche et al. 2018). Once samples are collected, enumeration can be carried out using microscopy or quantitative polymerase chain reaction (qPCR), and community composition and diversity can be tested using culture-dependent and culture-independent experiments. Culture-dependent studies give researchers the ability to study viable microorganisms collected from environmental samples by growing microorganisms on petri-dishes, but can only shed light on a very small portion of the community since most microorganism are not culturable in a laboratory setting. Culture-independent experiments depend on extraction of total DNA from environmental samples followed by gene amplification and sequencing, and provide the ability to identify thousands of microorganisms that culture-dependent experiments may miss. Sequencing techniques, however, do not afford the ability to distinguish between viable and non-viable microorganisms due to being able to detect fragments of DNA from non-viable microorganisms. Both culture-dependent and culture-independent studies rely heavily on effective and robust sample collection methods.

One of the current hurdles in aerobiology is the lack of a proficient sample collection method that overcomes the problems of cell desiccation during collection, low capture

rates for viral sized particles, and difficulties with purifying nucleic acids from samples collected [Griffin et al., 2010; Behzad et al., 2015]. Similar to other fields of microbiology, culture-dependent work has laid the foundation for understanding of the diversity of bioaerosols and was supplemented with the application of metagenomic sequencing to aerosol samples. Culture-dependent studies rely on a collection method that are able to collection a large and representative number of microorganisms over a short time during which the cells do not desiccate and remain viable. Culture-independent studies are typically based on DNA extraction and metagenomic sequencing, but the low biomass concentration of aerosol samples collected on filters makes DNA and RNA extraction challenging. Both culture and molecular based methods would benefit from the identification of a proficient collection method. Furthermore, the field would benefit from the standardization of methods because it would allow seamless comparison of results between studies.

Chapter 1. Aerosol Microbiome over the Mediterranean Sea; Diversity and Abundance

The Mediterranean Sea (MS) is an oligotrophic to ultra-oligotrophic basin and neighbors northern Africa, a major source of natural aerosols, and Europe, a source of anthropogenic aerosols. In my first chapter, I investigated 1) the controls on the diversity of airborne microbes, and 2) the source of the microbes comprising the aerosol microbiome over the Mediterranean Sea. Due to the proximity of the MS to land, I hypothesized that I would find high concentrations and high diversity of airborne

bacteria as land can be a major source of diverse bioaerosols. I further hypothesized that this community would encompass both marine and terrestrial microbes. In the manuscript, I described the abundance and genetic diversity of airborne bacteria in air samples collected over an East-West transect of the entire Mediterranean Sea. This manuscript was the first comprehensive biogeographical dataset to assess the diversity and abundance of airborne microbes over the Mediterranean Sea. The results shed light on the spatiotemporal distribution of airborne microbes over the ocean and may have implications for dispersal and distribution of microbes (biogeography) in the ocean (Mescioglu et al., 2019a)

Chapter 2. Impacts of bioaerosols on the biogeochemistry of the NRS

The northern Red Sea is an oligotrophic to mesotrophic marine ecosystem with high atmospheric deposition due to its proximity to arid regions, including northern Africa, the Middle East, and the Arabian Peninsula. In my second chapter, I tested the impacts of bioaerosols on the surface water microbial diversity and the primary and bacterial production rates in the Northern Red Sea (NRS) using a mesocosm bioassay experiment. The experiment was designed to answer 1) how the bioaerosols collected during dust-events impact prokaryotic and eukaryotic relative abundance in the NRS surface water, and 2) how the changes in community structure affect biogeochemical cycles of the NRS. By treating NRS surface seawater with dust, which contained nutrients, metals, and viable organisms, and "UV-treated dust" (which contained only nutrients and metals), I was able to assess the impacts of bioaerosols on local natural

microbial populations. Our results suggested that the airborne microorganisms and viruses alter the surface microbial ecology and primary and bacterial production rates of the NRS. Specifically, primary production was suppressed (as much as 50%), and bacterial production increased (as much as 55%) in the live dust treatments relative to incubations amended with UV-treated dust or the control. The diversity of eukaryotes was also lower in treatments with airborne microbes. These results may have implications for the carbon cycle in low nutrient marine ecosystems, which are expanding and are especially important since dust storms are predicted to increase in the future due to desertification and expansion of arid regions (Mescioglu et al., 2019b)

Chapter 3. Collection Efficiency of Airborne Microbes by Different Instruments: A Comparison Study

My third chapter tries to address the inconsistencies of bioaerosol collection instrumentation used in aeromicrobiology, which pose challenges for aeromicrobiologists, and can determine the success of a study. For this chapter, I tested the efficiency (number of colony-forming units, or CFUs, and DNA yield) of samples collected with three instruments: a membrane filtration device, a liquid impinger, and a portable electrostatic precipitator bioaerosol collector. The results show that the electrostatic precipitator collected microorganisms significantly more efficiently than the membrane filtration and liquid impingement in both types of studies over the same time interval, primarily due to its high flowrate. Using a sampler that can overcome technical hurdles can accelerate the advancement of the field, and the lightweight,

battery-powered, inexpensive, portable electrostatic precipitator bioaerosol collection device could address these limitations.

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Chapter One

AEROSOL MICROBIOME OVER THE MEDITERRANEAN SEA; DIVERSITY AND ABUNDANCE

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Abstract: Prokaryotic microbes can become aerosolized and deposited into new environments located thousands of kilometers away from their place of origin. The Mediterranean Sea is an oligotrophic to ultra-oligotrophic marginal sea, which neighbors northern Africa (a major source of natural aerosols) and Europe (a source of mostly anthropogenic aerosols). Previous studies demonstrated that airborne bacteria deposited during dust events over the Mediterranean Sea may significantly alter the ecology and function of the surface seawater layer, yet little is known about their abundance and diversity during 'background' non-storm conditions. Here, we describe the abundance and genetic diversity of airborne bacteria in 16 air samples collected over an East-West transect of the entire Mediterranean Sea during non-storm conditions in April 2011. The results show that airborne bacteria represent diverse groups with the most abundant bacteria from the Firmicutes (Bacilli and Clostridia) and Proteobacteria (Alphaproteobacteria, Betaproteobacteria, and Gammaproteobacteria) phyla. Most of the bacteria in our samples have previously been observed in the air at other open ocean locations, in the air over the Mediterranean Sea during dust storms, and in the Mediterranean seawater. Airborne bacterial abundance ranged from 0.7×10^4 to 2.5×10^4 cells m^{-3} air, similar to abundances at other oceanic regimes. Our results demonstrate that airborne bacterial diversity was positively correlated with the mineral dust content in the aerosols and was spatially separated between major basins of the Mediterranean Sea. To our knowledge, this is the first comprehensive biogeographical dataset to assess the diversity and abundance of airborne microbes over the Mediterranean Sea. Our results

shed light on the spatiotemporal distribution of airborne microbes over the ocean and may have implications for dispersal and distribution of microbes (biogeography) in the ocean.

1.1 Introduction

Prokaryotic microorganisms are found in the air over the global ocean in substantial numbers, with a median abundance of 6.7×10^3 m^{-3} air [1] and are referred to as 'airborne microbes'. These airborne microbes can originate from both land [2,3] and the ocean [4,5]. Upon aerosolization, wind can transport microbes over great distances, including across large ocean basins and seas [6–10]. The residence time of microbes in the air can reach up to seven days [1], which enables them to cross thousands of kilometers. While airborne, microbes are exposed to atmospheric oxidant gases [11] and meteorological factors, like temperature and UV [12], that can cause cell damage and reduce their viability. However, up to ~20% of these airborne microbes remain viable during atmospheric transport [13], and this has important implications for receiving ecosystems. Airborne microbes are deposited with dry (aerosol particles) or wet (rain) atmospheric deposition back onto Earth's surface, including the surface of the ocean [9,14,15].

Airborne microbes include a diverse array of organisms, and their deposition can impact human health through spreading infectious diseases [16], agriculture through dispersal of plant pathogens [17], and ecosystem productivity and function through introduction of new organisms [18]. Recently, it was shown that abundance of microbes in outdoor air can be influenced by seasons, with Bragoszewska and Pastuszka [12] reporting highest abundance in spring and Kaarakainen et al. [19] reporting highest abundance in the summer. Interestingly, certain bacterial species, like Streptomyces and Cladosporium, have stronger temperature and seasonal variation than other species,

like Penicillium and Aspergillus [19]. The diversity of microbes in outdoor air has also been shown to vary between environments [19], yet there are only a few studies investigating airborne microbes over oceans. The few studies conducted so far indicate that airborne microbes are found ubiquitously over marine environments, but their abundance, diversity, and the factors driving their diversity are still poorly studied [3,20].

The Mediterranean Sea (MS) is an ideal marine environment to study airborne microbes. The MS is a low-nutrient low-chlorophyll (LNLC) ecosystem [21,22], and the surrounding landmasses provide ample aerosols: The densely populated land to the north is a source of anthropogenic aerosols, and the arid land to the south is a source of mineral dust [20]. The effects of the high atmospheric deposition in this basin (1–50 g dust m^{-2} y^{-1} [23]) has been studied extensively and shown to be important chemically, providing limiting micro (e.g., Fe, Zn) and macro (e.g., N, P) nutrients to the MS [24–27]. In turn, these leached nutrients support primary production in the mixed layer of the MS [28] and can stimulate N_2 fixation, which may induce further primary production in the surface water [28,29]. In addition to leached nutrients and trace metals, atmospheric deposition has been shown to add viable microbes to the MS [30]. These airborne microbes can fix N_2 and utilize organic carbon (i.e., leucine) in seawater after deposition [30]. Therefore, airborne microbes may have an important contribution to the ecology of MS waters, with ecological implications for other LNLC settings receiving high atmospheric deposition, such as the North Atlantic Ocean.

Most studies investigating airborne microbes over the MS have focused on determining their diversity and abundance during dust storm events [20]. These studies showed that during storm events, airborne microbial abundance increases, and diversity is dependent on source [31,32]. However, it is also important to evaluate these variables during background conditions (clear days), which are far more common than dust storms events. Understanding background conditions may help identify what is unique about storm events that have resulted in measurable changes in the receiving water following deposition events [20]. Moreover, identifying airborne microbes and the factors driving their diversity over the ocean during background conditions may further our understanding of the mechanisms of bioaerosol dispersion, with possible implications for dispersal and biogeography.

In this study, we analyzed aerosol samples collected at all major basins of the MS (Levantine, Ionian Sea, Tyrrhenian Sea, Algero-Provencal basin, Alboran Sea) during "normal" background non-dust-storm conditions in April 2011 (spring). We analyzed the microbial diversity using 16S rRNA sequencing and microbial abundance using microscopy. Due to the proximity of the MS to terrestrial sources of aerosols, we hypothesized that we would find a high number of airborne bacteria comprising a diverse community. We further hypothesized that this community would encompass both marine and terrestrial microbes.

1.2 Methods and Materials

1.2.1. Sampling

Samples were collected aboard the R/V Meteor (cruise M84/3) during an east to west transect in the MS from 6th to 28th April 2011 (Figure 1). Aerosols were collected in all major basins onto Whatman 41 filters for 24 h using a high-volume sampler pumping air at 42 $m^3\ h^{-1}$ [30]. The sampler was positioned at the front of the ship (to reduce collection of ship emissions) and samples were processed in an aerosol designated laboratory. Volumes of air pumped, and the start and end coordinates of sample collection were recorded (Supplementary Table S1). The filters were frozen and kept at −80 °C until processing.

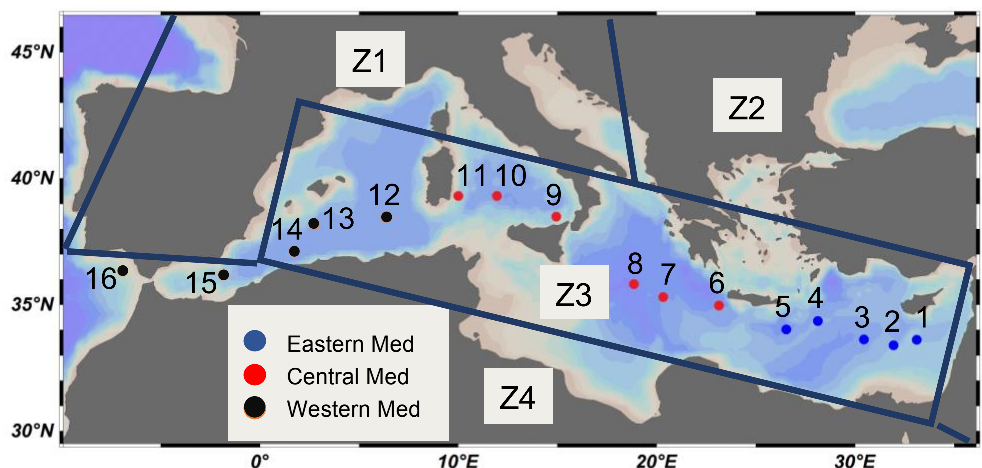

Figure 1.1. Map of the sites where aerosols were collected throughout the Mediterranean Sea (MS) in April on the R/V METEOR cruise M84/3, with sample IDs, region of collection (eastern Med = blue, central Med = red, and western Med = black), and airmass origin zones (Z1–Z4) shown.

1.2.2. Aerosol Optical Depth

To assemble additional information about the aerosols present over the MS at the time of sample collection, we used a global 1 × 1 degree and six-hourly 550 nm aerosol optical depth (AOD, an approximate measure of total atmospheric column of aerosol mass) reanalysis product that was developed and validated at the Naval Research Laboratory, CA, USA [33]. The core model of this aerosol reanalysis product is the Navy Aerosol Analysis and Prediction System (NAAPS), which characterizes anthropogenic and biogenic fine aerosol species (ABF, including pollutions from industry, fossil fuel and biofuel, and organic aerosols), dust, biomass-burning smoke and sea salt aerosols. The reanalyzed aerosol fields were obtained by running NAAPS with the assimilation of quality-controlled retrievals of AOD from moderate resolution imaging spectroradiometer (MODIS) on Terra and Aqua and the multi-angle imaging spectro radiometer (MISR) on Terra [34–36]. The fine and coarse mode AOD at 550 nm from the reanalysis is shown to have good agreement with the ground-based global scale sun photometer Aerosol Robotic Network (AERONET) observations regionally and seasonally [33]. Speciated AOD data were extracted (with the nearest neighbor method) from the NAAPS reanalysis along the ship track for the study period. Correlational relationships were analyzed between bacterial abundance and diversity and ABF, dust and total AODs, to compare to studies that have found increased abundances of bacteria associated with elevated pollution [37] and dust levels [38].

1.2.3. Air Mass Backward Trajectories

Seventy-two-hour isentropic back trajectories were constructed from the National Oceanic and Atmospheric Administration (NOAA) database using the hybrid single-particle Langrangian integrated trajectories (HYSPLIT) program [39]. Back trajectories for elevations 50, 250, and 500 m were computed using the GPS coordinates of the midpoint between the start and end locations of sampling for each filter (Supplementary Table S1, Supplementary Figure S1). The samples were assigned to one of four origin zones according to the geographic location from which the airmass originated, as determined from the backward trajectory model results (Table 1, Supplemental Figure S1). The four zones are Western Europe (Z1), Mediterranean Sea (Z2), northern Africa (Z3), Eastern Europe (Z4) (Figure 1). Note that in some cases the airmass crossed more than one zone during collection (Supplemental Figure S1).

1.2.4. Region and Distance to Land

Samples were grouped according to the location of collection (Figure 1, Table 1) in order to determine if the diversity was influenced by location and if proximal sites had similar diversity. We also measured the distance from the closest landmass, including islands, at five points of sampling (beginning, quarter-point, midpoint, three-quarters point, and end) for each sample, and used the average of the five values as the distance from land in our analysis (Table 1).

Table 1.1. Sample ID, region of the MS samples were collected from, airmass origin of samples based on HYSPLIT back trajectory models (Z1 = Western Europe, Z2 = Mediterranean Sea, Z3 = Northern Africa, and Z4 = Eastern Europe), distance between the sampling site to the closest landmass or island (km), total aerosol optical depth (AOD), aluminum concentrations (ng m^{-3} air), number of OTUs observed, and Shannon's Diversity Index (H) are shown for each sample.

Sample ID	Region	Airmass Origin	Distance from Land (km)	Total AOD	Aluminum (ng m^{-3} air)	Observed OTUs	Shannon's Index (H)
1	Eastern	Z2	155	0.282	569	241	7.48
2	Eastern	Z3	154	0.285	516	158	6.76
3	Eastern	Z3	102	0.22	331	100	6.06
4	Eastern	Z3	204	0.224	410	141	6.41
5	Eastern	Z1	156	0.196	205	119	5.94
6	Central	Z1	78	0.233	220	95	5.75
7	Central	Z1	213	0.225	661	96	6.03
8	Central	Z1	288	0.133	404	92	5.89
9	Central	Z2	48	0.18	134	66	5.17
10	Central	Z2	112	0.115	41	76	5.64
11	Central	Z1	49	0.147	104	96	6.04
12	Western	Z4	135	0.359	196	164	6.83
13	Western	Z4	126	0.252	172	82	5.83
14	Western	Z4	56	0.134	395	70	5.7
15	Western	Z4	36	0.2	355	108	6.37
16	Western	Z1	42	0.11	103	75	5.79

1.2.5. Al

After collection, a subsample of the Whatman 41 filters was dried in a desiccator for 24 h before being reweighed. Filters were digested with hydrogen fluoride (HF) following the procedure of ASTM (1983) [27]. Al was measured on an atomic absorption spectrometer Agilent 280FS AA and graphite furnace Agilent 240Z AA.

1.2.6. Bacterial Abundance

Subsamples from each of the filters were cut with sterile scissors (3 × 3 cm), placed into 5 mL of sterile MS water, and fixed with ultrapure glutaraldehyde solution (Sigma, St. Louis, MO USA, final concentration 0.02% *v:v*). The filters were sonicated for 5

min to detach organisms from the filter, stained with SYBR green solution (Applied Biosystems, Foster City, CA USA), and filtered through a 0.2 μm polycarbonate filter (PALL). The filters were placed on a microscope slide, and bacterial cells were enumerated using epifluorescence microscopy (Olympus BH12). The values were normalized to the area of the whole filter (17 × 23 cm) and divided by the volume of air pumped during collection to determine the number of cells per m^3 of air. SYBR green is a robust bacterial stain [40] used in numerous microbiology studies, including aeromicrobiology studies [1,41]. Further, we used microscopy-grade SYBR, so the introduction of counting errors is unlikely.

1.2.7. DNA Extraction, Amplification, Sequencing

Subsamples from each of the filters were cut with sterile scissors (2 cm × 2 cm), and total DNA was extracted in triplicates using the phenol chloroform method, modified from Massana et al. [42]. The triplicates were pooled into one sample to ensure enough DNA for sequencing. The DNA was sent to Mr. DNA Molecular Research Laboratories. Polymerase chain reaction (PCR) using primers 515 (forward) and 806 (reverse) to amplify 16S rRNA, with barcodes on the forward primer, were carried out using the HotStarTaq Plus master mix kit (Qiagen, Valencia, CA USA). The conditions of the protocol were as following: 94 °C for 3 min, 28 cycles of 94 °C for 30 s, 53 °C for 40 s and 72 °C for 1 min, and a final elongation step at 72 °C for 5 min. PCR products were visualized in 2% agarose gel using electrophoresis to confirm successful amplification. The samples were pooled together in equal proportions (based on their MW and DNA concentrations), purified using calibrated Ampure XP beads,

and used to prepare libraries using a Nextera DNA sample preparation kit (Illumina, Foster City, CA USA). Libraries were loaded to a 600 cycles v3 reagent cartridge (Illumina) and the sequencing was performed on Miseq (Illumina). DNA extraction and amplification protocols were repeated for blank filters brought onboard the cruise and treated similarly to the samples, and the PCR products were checked by electrophoresis. The electrophoresis visualization showed no amplification bands indicating there was no contamination by the filters (i.e., no microbes present on the filters).

1.2.8. Bioinformatics

Samples were processed using the open-source Quantitative Insights into Microbial Ecology 2 (QIIME 2) pipeline [43]. Sequences were demultiplexed and barcodes were trimmed using the cutadapt plugin [44]. Data were denoised using dada2 [45], sequences were clustered into amplicon sequence variants (ASVs) which can be thought of as 100% operational taxonomic units (OTUs). Taxonomic classifier was trained [46] using Greengenes [47]. Taxonomies were assigned using the Naive Bayes method [48]. Samples were filtered to remove sequences identified as mitochondria and chloroplast. Alpha-diversity metrics (observed OTUs and Shannon's diversity index [H] [49], beta diversity metrics (weighted UniFrac [50]), and principle coordinate analysis (PCoA) were estimated using q2-diversity after samples were rarefied (subsampled without replacement). The samples were grouped according to the location in which they were collected in the MS (i.e., region) (Figure 1) and the origin of the air mass (Figure 1) to test how abundance, richness, and diversity were

influenced by these factors. Weighted UniFrac distances (a quantitative measure of community dissimilarity that incorporates phylogenetic relationships between the bacteria) were used to generate the PCoA plots (Figure 6A,B). Associations between regions of sample collection and UniFrac were tested using PERMANOVA (Figure 6A) [51] to investigate whether microbial communities in samples within a region (e.g., Eastern MS) were more similar to each other then they were to samples from the other regions (e.g., Central MS and Western MS). We also tested for any association between geographical distances and community dissimilarity (weighted UniFrac) (Figure 6B) using the Mantel test. To simplify visualization of relative abundance, we clustered bacteria into two categories based on their relative abundance in our samples: (1) "Common" bacteria (families that made up more than 5% of at least 1 sample) (Table 2), and (2) "rare" bacteria which did not meet the 5% relative abundance threshold (Table 3).

1.3. Results and Discussion

1.3.1. Aerosol Origin and Chemical Properties

The aerosol optical depth (AOD) data derived from the Navy Aerosol Analysis and Prediction System (NAAPS) AOD reanalysis, as described in the methods section are shown in Figure 2. Total AOD, which includes mineral dust, anthropogenic and biogenic fine aerosol species (ABF), smoke, and sea salt, during the cruise ranged from 0.11 to 0.36, with the lowest values measured during collection of sample 16 and the highest measured during collection of sample 12, both collected from the Western MS

(Figures 1 and 2, Table 1). ABF and mineral dust were the main contributors to the total AOD during our study, together comprising between 60% and 88% of total AOD. Smoke and sea salt estimates from the NAAPS model were both relatively low in concentration and evenly distributed in all the samples. Smoke and sea salt contributed only to a small portion of total AOD during our sampling period, and thus were not included in further analysis.

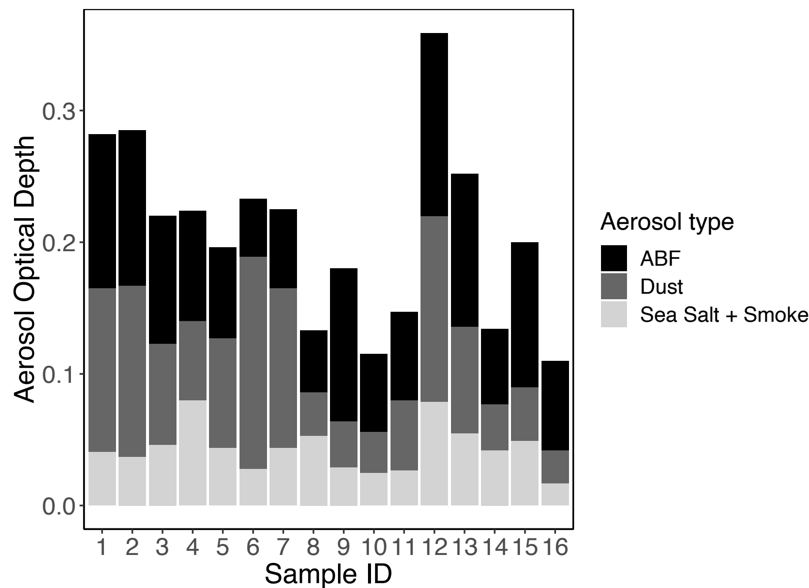

Figure 1.2. Aerosol optical depth (AOD) from Navy Aerosol Analysis and Prediction System (NAAPS) reanalysis at the time of collection at sample sites. Each bar represents one sample, with the height of each bar corresponding to total AOD. ABF (anthropogenic and biogenic fine aerosol species) in black, mineral dust in dark gray, and sea salt + and smoke in light gray fractions of the total AOD are shown for each station.

The average AOD fraction, based on NAAPS reanalysis, attributed to dust in the MS during the month of April for 2003–2018 was on the order of 0.1–0.2 with a decreasing gradient from the south (closer to the African continent, the main aerosol source) to the north, and generally decreasing from east to west. However, this long-term average for April likely included some dust-storm events. During our sampling

(April 2011) dust AOD on April 7–8, (samples 1 and 2), April 12–13 (samples 6 and 7) and April 18 (sample 9) was relatively high compared to other days (Figure 2). However, dust AOD for these days was still low compared to dust contribution to AOD during storms, which can frequently exceed 1.0 [52,53]. From the low-level wind and the movement of dust plumes based on NAAPS reanalysis and NOAA HYSPLIT back trajectories (Supplementary Figure 1), dust detected at the location of the ship on April 7–8 (samples 1 and 2) likely originated from Turkey. The April 12–13 (sample 6 and 7) dust peak observed is related to a dust storm that occurred in the northwest of Africa on April 5 (with maximum dust AOD around 2.0) [52]. NAAPS reanalysis shows that the dust plume originating from this storm moved northwest and reached 60° N on April 9 and then moved southeastward and reached the location of the ship on April 12. After this long-range transport, dust AOD was much weaker when it arrived at the MS (0.14). As this air mass traveled over the European continent, it mixed with anthropogenic aerosols (ABF). Throughout the cruise, ABF ranged from 0.04 to 0.14, with the lowest ABF AOD during collection of sample 6, in the central MS, and the highest during collection of sample 12 in the western MS (Table 1, Figure 2).

Aluminum (Al) concentration, a proxy for mineral dust [27], ranged between 41 and 661 ng m^{-3} air, and was highest on April 13 during collection of sample 7, which occurred when the dust storm originating from northwest Africa arrived in the MS. Overall, Al measurements were positively correlated with total AOD (Spearman correlation: $\rho = 0.694$, $p = 0.004$), and especially with the AOD fraction attributed to dust (Spearman correlation: $\rho = 0.834$, $p < 0.0001$) (Table 1). There was also a

significant positive correlation of Al concentrations and longitude, with more Al in samples collected in the air above the Eastern MS than above the Western MS (Spearman correlation: $\rho = 0.597$, $p = 0.017$) (Table 1).

Overall, the aerosol concentration in the air during our sampling campaign (background non-dust-storm period), particularly the mineral dust (as derived from the dust fraction of AOD and Al), were within the lower range of previously measured values in days without dust storms and about an order of magnitude lower than values recorded during dust storm event in the region [52,53].

1.3.2. Airborne Bacterial Abundance

Bacterial abundances in our samples ranged from 10^3 to 10^4 cells m^{-3} air (Figure 3). The highest abundance of bacteria was measured in sample 6 in the Central MS (2.12×10^4 cells m^{-3} air), near the island of Crete, during the arrival of the tail of the dust storm that originated from North Africa. The lowest abundances were measured in the Central and Eastern Mediterranean (6.64×10^3 to 7.17×10^3 cells m^{-3} air) in samples 9 and 4, respectively. Bacterial abundances in aerosols collected over the MS were in agreement with previous studies from the eastern Mediterranean coast [10] and the Atlantic, Pacific, and Indian ocean basins [1], yet were lower than those reported in the East China Sea [54] and the Red Sea [55]. Rahav et al. [10] measured the abundance of airborne prokaryotes at a coastal site located at the easternmost MS during 34 sampling events (between 2015 and 2018) and found that abundances were positively correlated to the concentration of aerosols in the air (mg m^{-3} air). Here, however, we did not find such a correlation, likely because the range of concentrations during non-

dust-storm conditions, represented by our samples, was relatively small in comparison to previous studies. Mayol et al. [1] measured bacterial abundances in the Atlantic, Indian, and Pacific Ocean basins and found that sites closer to land (including islands) had significantly higher numbers of airborne microbes (normalized to the aerosol mass) than those further away from land-masses. This was also not observed in the MS, possibly because the MS is surrounded by land, and all sampling sites are relatively close to land when compared to samples obtained in the open ocean by Mayol et al. [1].

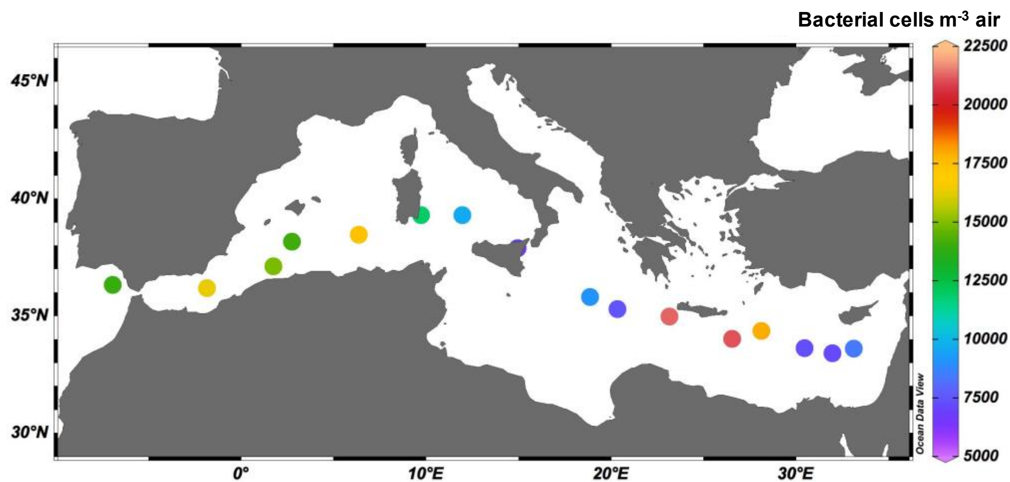

Figure 1.3. Spatial distribution of airborne bacterial abundance (cells m^{-3} air) over the MS during April 2011.

1.3.3. Airborne Microbiome above the MS

Fifty-nine unique families of bacteria were found in the samples collected during this study. The relative abundance was used to group bacteria into two categories:

“Common” (Table 2) or “rare” (Table 3). Families that had a relative abundance of 5% or greater in at least one of our samples were considered “Common”, and families that did not meet the 5% threshold were considered “rare”. Common bacteria in our samples belonged to five phyla: Actinobacteria (three families), Bacteroidetes (two families), Firmicutes (eight families), Proteobacteria (eleven families), and Deinococcus-Thermus (one family) (Table 2). These bacteria are of variable gram stains, have diverse oxygen requirements, spore formation, and come from many different habitats (Supplementary Table S2). Five bacteria in our samples, *Chitinophagaceae sediminibacterium, Clostridiaceae SMB53, Veillonellaceae* spp.*, Moraxellaceae acinetobacterlwoffii*, and *Sinobacteraceae* spp., had not previously been reported in aerosol samples. All other organisms have previously been identified in airborne bacterial studies in different locations around the world (Supplementary Table S2) and may represent the consortium of bacteria that are more likely to be aerosolized, transported long distance, and hence dispersed over large areas.

Table 1.2. 'Common' organisms found over the Mediterranean Sea in this study compared to five studies focusing on marine aerosols [1,8,38,55,56], five studies focusing on coastal aerosols [3,31,32,57,58], and six samples from one study focusing on Mediterranean surface seawater [59]. Columns under open ocean studies refer to references [1,8,38,55,56], columns under Mediterranean coastal studies refer to references [3,31,32,57,58], and columns under Mediterranean seawater samples refer to six samples from reference [59].

Common Bacteria				Open Ocean Studies					Med Coastal Studies					Med Seawater Samples					
Phyla	Class	Order	Family	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	6
Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	×															
Actinobacteria	Actinobacteria	Actinomycetales	Corynebacteriaceae	×					×	×									
Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	×															
Bacteroidetes	Flavobacteriia	Flavobacteriales	Weeksellaceae	×															
Bacteroidetes	Saprospirae	Saprospirales	Chitinophagaceae						×		×	×							
Firmicutes	Bacilli	Bacillales	Bacillaceae	×	×	×		×	×	×	×	×			×	×	×	×	×
Firmicutes	Bacilli	Bacillales	Paenibacillaceae	×				×											
Firmicutes	Bacilli	Bacillales	Planococcaceae	×				×	×										
Firmicutes	Bacilli	Bacillales	Staphylococcaceae	×	×				×		×								
Firmicutes	Bacilli	Turicibacterales	Turicibacteraceae	×															
Firmicutes	Clostridia	Clostridiales	Clostridiaceae						×				×						×
Firmicutes	Clostridia	Clostridiales	Peptostreptococcaceae																
Firmicutes	Clostridia	Clostridiales	Veillonellaceae																
Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	×		×			×						×	×	×	×	
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	×			×				×	×		×	×	×	×	×	×
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	×										×	×	×	×	×	×
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	×	×	×		×	×		×		×				×		
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	×										×	×	×	×	×	×
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	×				×					×	×	×	×	×	×	×
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	×		×		×	×				×						
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	×	×	×		×				×		×		×	×	×	
Proteobacteria	Gammaproteobacteria	Salinisphaerales	Salinisphaeraceae	×															
Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	×			×							×	×	×	×		
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	×															
Deinococcus-Thermus	Deinococci	Thermales	Thermaceae													×			

The relative abundances of the "common" orders of bacteria in each region of the MS (Eastern, Central, Western) are shown in a bar plot (Figure 4), with rare bacteria (constituting less than 5% of all samples) grouped into "other". The Eastern MS had a higher relative abundance of *Bacillales, Salinisphaerales,* and *Enterobacteriales*, and lower relative abundances of *Clostridiales* and *Saprospirales* than the Western and Central regions (Figure 4). The most abundant bacteria found over the MS were Firmicutes (Bacilli and Clostridia) and Proteobacteria (Alphaproteobacteria, Betaproteobacteria, and Gammaproteobacteria) (Figure 4). The Firmicutes and Proteobacteria families we found over the MS have previously been isolated from variable habitats, including soil, plant microbiota, aquatic (including marine) and thermal environments, and human and animal microbiota (Figure 4, Supplementary Table S2). This suggests that the bacterial community of the MS air during non-storm conditions are not tied to one habitat source. The organisms that were significantly more abundant during higher concentrations of dust (Bacillaceae, Paenibacillaceae) are both from the Bacillales order and are terrestrial microbes, commonly found in soil and plant microbiomes (Supplementary Table S2). This is consistent with data from coastal Mediterranean aerosol studies conducted during dust storms [3,31,32,57,58], which also reported the presence of Bacillaceae in the air during storm events. Certain bacteria were more abundant in samples with high concentrations of ABF (Chitinophagaceae, Staphylococcaceae, Planococcaceae, Turicibacteraceae). However, these organisms are found in a wide array of habitats, and thus implication of their association to high concentrations of ABF is not as clear.

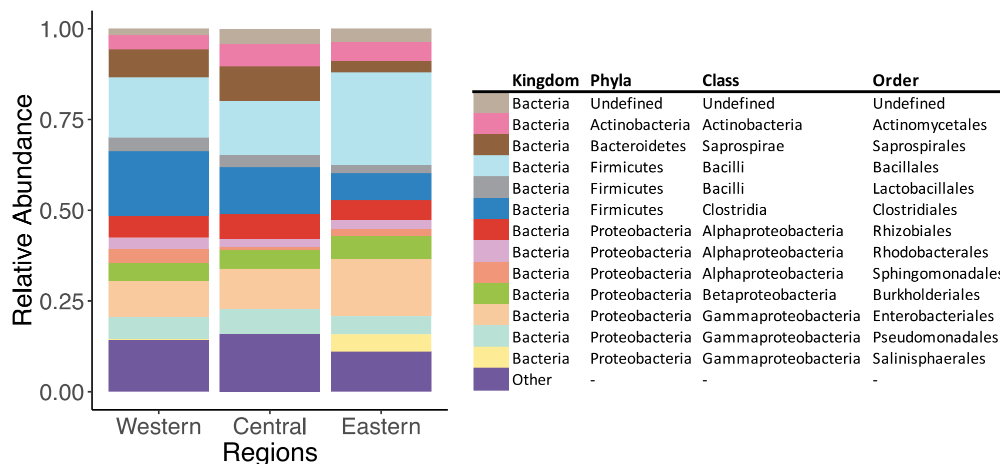

Figure 1.4. Relative abundance of prokaryote operational taxonomic units (OTUs) in the different regions of the MS. The colors correspond to different taxonomic orders of prokaryotes, as shown in the detailed legend.

To quantitatively assess the diversity and estimate the differences in airborne bacterial communities over the MS, we report microbial community richness, expressed as the number of unique OTUs observed, and diversity expressed as Shannon's diversity index (H), estimated from the abundance of bacteria in each sample (Table 1). Observed OTUs corresponds to the number of unique bacteria in each sample, whereas H is a commonly used quantitative measure of diversity [49]. The abundance of observed OTUs ranged from 66 (sample 9) to 241 (sample 1) (Table 1). Observed OTUs varied significantly between the three regions of the MS: Eastern (median = 141), Central (median = 93), and Western (median = 82) (Kruskal–Wallis pairwise test: H = 6.732, df = 2, p = 0.034) (Table 1) and correlated positively to Al concentration (Spearman correlation: ρ = 0.549, p = 0.028), mineral dust AOD (Spearman correlation: ρ = 0.68, p = 0.003), ABF AOD (Spearman correlation: ρ = 0.538, p = 0.031), and total AOD (Spearman correlation: ρ = 0.70, p = 0.002) (Table

1). The diversity ranged from 5.17 (sample 9) to 7.48 (sample 1) (Table 1). H values were positively correlated to mineral dust (Spearman correlation: $\rho = 0.547$, $p = 0.028$), ABF (Spearman correlation: $\rho = 0.599$, $p = 0.014$, and total AOD concentrations (Spearman correlation: $\rho = 0.653$, $p = 0.007$) (Figure 5). Prokaryotic communities from samples within the Eastern MS were significantly more similar to each other than samples from the Western MS (PERMANOVA: F = 1.83, $p = 0.009$) (Figure 6A). Moreover, distance to land, including islands, was positively correlated to community similarity (Spearman: $\rho = 0.377$, $p = 0.009$) (Table 1, Figure 6B), even though the bacterial abundance did not correlate to distance to shore.

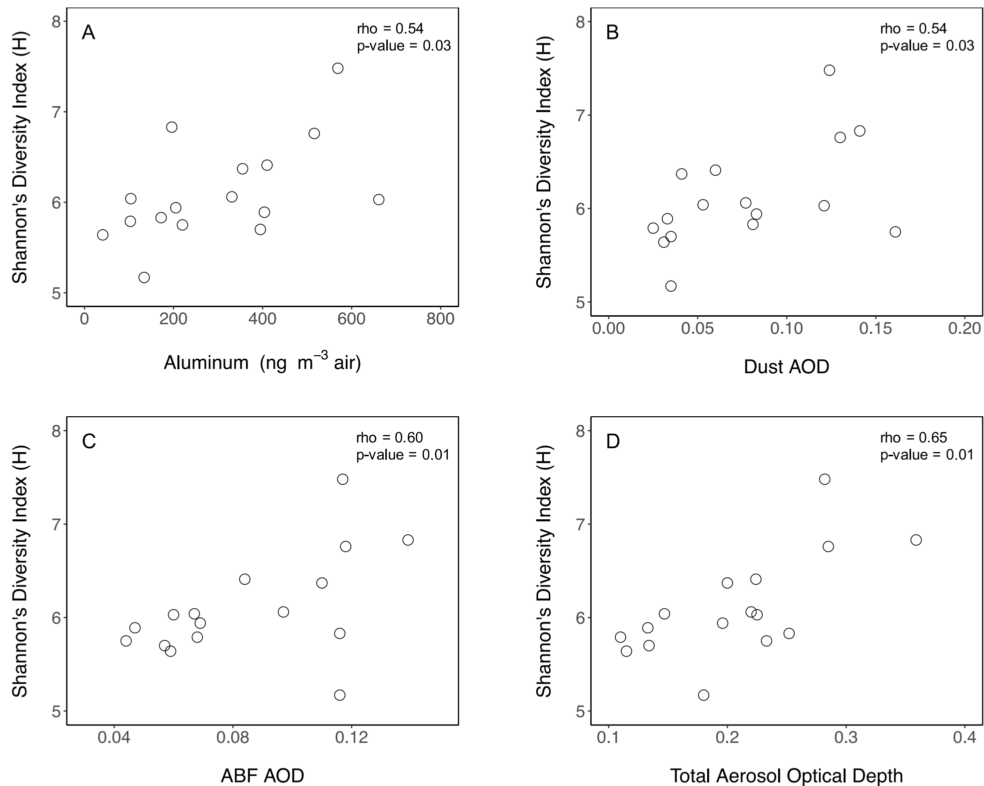

Figure 1.5. The relationship between bacterial diversity (Shannon's diversity index) and atmospheric aerosols variables, (**A**) aluminum, (**B**) dust, (**C**) ABF, (**D**) total aerosol optical density.

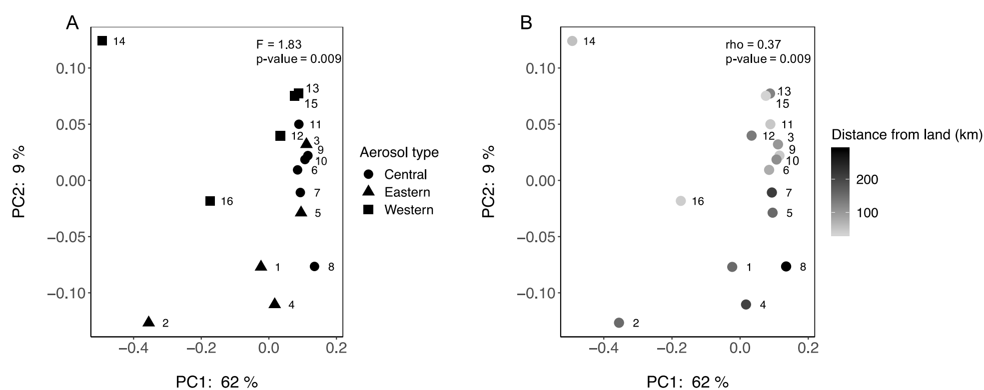

Figure 1.6. PCoA showing the differences in beta diversity using weighted UniFrac with (**A**) shapes representing regions of the MS samples were collected in, and (**B**) distance (km) between each sample site and closest landmass.

Previous studies conducted during different dust storms have shown that the origin and atmospheric route of airmass influences bacterial community composition [30,54]. Our findings show that, during non-dust-storm events, neither bacterial richness nor diversity are influenced by the origin of the airmass. This is likely because during intense dust events copious amounts of desert topsoil from different locations were transported and these topsoil particles had distinct microbial communities [32,60]. Our study took place during non-storm conditions over the ocean, and hence terrestrial origin signatures were less pronounced. Instead, we show that airborne bacterial richness and diversity varied by geographic location over the MS (not the origin of the airmass) (Table 1) and correlated to the concentration of Al (Table 1) (as well as dust AOD, ABF AOD, and total AOD; Table 1, Figure 2). Similarly, the diversity of airborne microbes over the MS increased with increasing concentration of Al, mineral dust AOD, ABF AOD, and total AOD measurements (Table 1, Figure 5).

Microbes in the air are predominantly associated with particles, hence when there more particles in the air, it is likely to encounter more bacteria. Interestingly, it has

been suggested that crevices in particles maintain local humidity due to moisture adsorption to particles and provide shelter from UV, thus protecting airborne microbes from desiccation and exposure to damaging UV radiation, two elements reducing survival of bacteria in the atmosphere [61–63]. Dust may also increase the survival potential of airborne bacteria because dust particles can scatter light and UV radiation, reducing exposure. Thus, high Al, which indicates more mineral dust particles (Figure 5), results in an increased diversity of airborne microbes (Figure 5). This may be because there are more unique OTUs when there are more mineral dust particles in the air since mineral dust has higher microbial diversity compared to anthropogenic pollutant sources. Alternatively, the chance of encountering more unique OTUs in the air may increase when there are more dust particles in the air because sampling, DNA extraction, PCR, and sequencing methods are better at detecting "rare" OTUs under such conditions. The correlation between mineral dust and diversity suggests that the microbiome of the air will become more diverse with increased desertification and related dust input to the atmosphere due to predicted changes in climate.

Our samples contained a high percentage (44%) of bacteria that are also found in MS surface water [61]. Additionally, we found that weighted UniFrac, (a measure of beta diversity) positively correlated to distance from land, including islands, regardless of the landmass type (island, populated, un-populated, desert or vegetated) (Table 1, Figure 6B). Although this correlation was rather weak, it may be explained by samples far from land containing a higher proportion of marine prokaryotes, in agreement with Mayol et al. [1]. Waves and bubble bursting in the sea surface also result in the

aerosolization and transportation of microbes [64]. Indeed, other open ocean aerosol studies have also identified marine bacteria in aerosols [1,54]. Our study demonstrates that aerosolization can be a mechanism for long-distance dispersal for marine bacteria [54,64]. This can have ecological implications for receiving ecosystems and may impact the biogeography of various strains. Airborne microbes can change the community structures of environments into which they are deposited [65,66]. Furthermore, bacteriophages associated with marine bacteria can also be transported to new environments and spread viral infections [66,67]. Therefore, airborne microbes and viruses may impact both microbial community structure and microbial production and should be further studied.

The average number of OTUs in our aerosol samples collected during springtime was similar to the number of OTUs in the Norwegian Sea and the Western Pacific in the summer and lower than OTUs in the Northern and Western Pacific Ocean in the fall [56]. Since seasonality impacts airborne bacterial abundance [12,19] and community composition [56], spatiotemporal variability of airborne microbes should be studied during other seasons to assess interannual variability in this region. The number of observed OTUs during non-storm conditions was lower than those measured in coastal cities in the Mediterranean during dust storms [31,58]. This is likely due to the positive correlation between the concentrations of various aerosol constituents (mineral dust and ABF) and the number of OTUs as observed in our study (Figure 5) and previous studies [31].

To our knowledge, there are only two other studies of airborne microbes in the MS during non-storm conditions [10,32]. The study site of Gat et al. [32] was a coastal city in Israel (~10 Km away from the shoreline) and the study site of Rahav et al. [10] was the rooftop of a building directly next to the ocean. Thus, these studies represent different ecological systems than the open ocean. However, all of the organisms that were prominent during clear non-storm days in Gat et al. [32] were also found in our samples (aside from Dermabacteraceae [Actinobacteria]) indicating that they are commonly in the air over both the land and the water in this region. Several other studies have reported on the airborne bacterial communities during dust storms in the MS and found organisms that were also present in our samples (Table 2) [3,31,32,57,58], suggesting that some organisms previously assumed as being dust-associated exist over the MS during non-storm conditions as well. Some organisms observed during dust storms, however, were absent during non-storm conditions [3,31,32,57,58], particularly many that are ubiquitously found in soils [60].

There are only a few studies which have identified and reported airborne microbial diversity in open ocean settings. However, the few reports cover diverse ocean basins, including the East China Sea [54], Caribbean Sea [8], Norwegian Sea [56], Atlantic Ocean [1,8], Pacific Ocean [1,56], and the Indian Ocean [1]. All these studies identified organisms at the family level, except for Mayol et al. [1], which identified organisms at the class level. We compared the microbes found in our study to organisms found in other open ocean studies (Tables 2 and 3) and found that 44% of the most common bacteria in our study were also reported in other open ocean aerosol studies at the family

level. We also found that 80% of the bacteria at the class level were found in aerosols in other marine studies. Of the rare bacteria (<5% in our samples), 16% were reported in other open ocean aerosol studies at the family level and 60% at the class level (Table 2).

Table 1.3. ‘Rare’ organisms found over the Mediterranean Sea in this study compared to five studies focusing on marine aerosols [1,8,38,55,56], five studies focusing on coastal aerosols [3,31,32,57,58], and six samples from one study focusing on Mediterranean surface seawater [59]. Columns under open ocean studies refer to references [1,8,38,55,56], columns under Mediterranean coastal studies refer to references [3,31,32,57,58], and columns under Mediterranean seawater samples refer to six samples from reference [59]

Rare Bacteria				**Open Ocean Studies**					**Med Coastal Studies**					**Med Seawater Studies**					
Phyla	Class	Order	Family	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	6
Actinobacteria	Actinobacteria	Actinomycetales	Dietziaceae	×															
Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae	×	×		×		×	×			×						
Actinobacteria	Rubrobacteria	Rubrobacterales	Rubrobacteraceae						×				×						
Bacteroidetes	BME43	Unassigned	Unassigned																
Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae																
Bacteroidetes	Cytophagia	Cytophagales	Amoebophilaceae	×															
Bacteroidetes	Saprospirae	Saprospirales	Undefined																
Bacteroidetes	Saprospirae	Saprospirales	Saprospiraceae																
Chlamydiae	Chlamydiia	Chlamydiales	Undefined																
Chlamydiae	Chlamydiia	Chlamydiales	Rhabdochlamydiaceae																
Cyanobacteria	4C0d2	MLE112	Unassigned												×			×	×
Firmicutes	Bacilli	Bacillales	Unassigned	×											×		×	×	
Firmicutes	Bacilli	Bacillales	Alicyclobacillaceae	×															
Firmicutes	Bacilli	Bacillales	Thermoactinomycetaceae	×															
Firmicutes	Bacilli	Bacillales	Exiguobacteraceae	×															
Firmicutes	Bacilli	Gemellales	Gemellaceae	×				×											
Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	×															
Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	×									×						

Firmicutes	Clostridia	Clostridiales	Tissierellaceae																
Proteobacteria	Undefined	Unassigned	Unassigned																
Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	×															
Proteobacteria	Alphaproteobacteria	Rhizobiales	C-	×															
Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylocystaceae	×															
Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	×										×	×	×	×	×	×
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Hyphomonadaceae	×											×				
Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	×		×		×		×	×	×							
Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	×			×							×	×	×	×	×	×
Proteobacteria	Gammaproteobacteria	Oceanospirillales	Halomonadaceae	×			×												
Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	×															
Spirochaetes	Spirochaetes	Spirochaetales	Spirochaetaceae																
WPS	Unassigned	Unassigned	Unassigned																
Deinococcus-Thermus	Deinococci	Deinococcales	Deinococcaceae																

When compared to airborne bacteria in samples collected on the Mediterranean coast [3,31,32,57,58] (Tables 2 and 3), 48% of the common bacteria and 13% of the rare bacteria found in our study were also reported in these studies at the family level. Highly abundant families (Bacillaceae, Sphingomonadaceae, and Pseudomonadaceae) were also found in the air over other marine environments [1,8,56] at the class level, suggesting that these organisms are commonly dispersed via airmasses. If these organisms are viable upon deposition and have a cosmopolitan distribution throughout the oceans, it could be inferred that airmasses are a vehicle of biogeographical distribution.

Mediterranean seawater samples contained the Bacillaceae family, as well as nine other bacterial families from the Proteobacteria (Bradyrhizobiaceae, Rhodobacteraceae, Rhodospirillaceae, Sphingomonadaceae, Comamonadaceae, Enterobacteriaceae, Pseudomonadaceae, Vibrionaceae) and Deinococcus-Thermus (Thermaceae) phyla. Vibrionaceae and Alteromonadaceae families, which were present in our as well as other studies, have most commonly been found in the sea surface microlayer [68], the ~100 μm surface layer of the ocean where there is a dynamic exchange between the sea and air [69]. Overall, 44% of common airborne bacteria and 16% of rare airborne bacteria in our study were previously reported to be found in the MS surface water [61] (Tables 2 and 3). The large proportion of organisms being found in both air and water as opposed to air only suggests that the bacterial exchange between sea and air during ‘normal’ atmospheric conditions is an important process that can influence the community structure of both environments.

Current data show a wide range of biogeochemical responses related to atmospheric deposition events in LNLC areas [20]. However, the specific contribution of airborne microbes to the changes documented in these studies is typically not considered [3,33]. To predict the future of LNLC regions and how they will contribute to global biogeochemical cycles, it is imperative to understand how atmospheric deposition impacts these regions [20], and to specifically determine the contribution of airborne microbes to these impacts.

The prevalence and importance of airborne microbes is clear, but key methods in aeromicrobiology have not yet been standardized (sample collection, quantification). We used filters to collect aerosols, but different studies have used other techniques, such as liquid impingement [70–72] or electrostatic precipitation [73–75]. Similarly, we measured bacterial abundance directly from filters after sonication to promote detachment from the filter, while others used different methods, such as qPCR [54], culture-based methods [3] and flow cytometry [10]. As a result, it is difficult to reliably compare results between studies, even if the sampling site and environmental conditions are similar. These issues merit further research and would provide meaningful advancements to the field.

1.4. Conclusions

Our results show that a diverse array of microbes is present in the air over the MS, with abundances similar to those over other ocean settings. We found that the diversity of the airborne microbes over the MS during non-dust-storm conditions is influenced by aerosol content (mineral dust as well as polluted aerosols) in the air. Our results also

show high percentages of marine bacteria in the air, indicating that there is a significant exchange of microbes between the sea surface and the air, even during background non-storm conditions. We also note that several groups of bacteria are more commonly found in the air, hence these groups may be readily dispersed by air movement with implications to their biogeography. Since desertification may increase with climate change, more particles will be introduced to the air, increasing the abundance and diversity of airborne microbes. This may have a significant impact on the microbial communities and biogeochemical cycles of oceans, particularly in regions that are subject to high rates of atmospheric deposition.

1.5 Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Figure S1: Backward trajectories constructed using NOAA HYPSPLIT MODEL for each sample, Table S1: Metadata of Samples, Table S2: Detailed Description of Common Bacteria.

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Chapter Two

DUST-ASSOCIATED AIRBORNE MICROBES AFFECT PRIMARY AND BACTERIAL PRODUCTION RATES, AND EUKARYOTIC DIVERSITY, IN THE NORTHERN RED SEA: A MESOCOSM APPROACH

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Abstract: The northern Red Sea (NRS) is a low-nutrient, low-chlorophyll (LNLC) ecosystem with high rates of atmospheric deposition due to its proximity to arid regions. Impacts of atmospheric deposition on LNLC ecosystems have been attributed to the chemical constituents of dust, while overlooking bioaerosols. Understanding how these vast areas of the ocean will respond to future climate and anthropogenic change hinges on the response of microbial communities to these changes. We tested the impacts of bioaerosols on the surface water microbial diversity and the primary and bacterial production rates in the NRS, a system representative of other LNLC oceanic regions, using a mesocosm bioassay experiment. By treating NRS surface seawater with dust, which contained nutrients, metals, and viable organisms, and "UV-treated dust" (which contained only nutrients and metals), we were able to assess the impacts of bioaerosols on local natural microbial populations. Following amendments (20 and 44 h) the incubations treated with "live dust" showed different responses than those with UV-treated dust. After 44 h, primary production was suppressed (as much as 50%), and bacterial production increased (as much as 55%) in the live dust treatments relative to incubations amended with UV-treated dust or the control. The diversity of eukaryotes was lower in treatments with airborne microbes. These results suggest that the airborne microorganisms and viruses alter the surface microbial ecology of the NRS. These results may have implications for the carbon cycle in LNLC ecosystems, which are expanding and are especially important since dust storms are predicted to increase in the future due to desertification and expansion of arid regions.

2.1. Introduction

Aerosols impact marine ecosystems by delivering macro- and micronutrients to surface seawater upon deposition [1–3]. These nutrients typically induce an increase in phytoplankton abundance and bacterial biomass and activity [4–7]. Atmospheric deposition also supplies a diverse array of microbes to marine ecosystems [8–10], of which up to 25% remain viable [11]. Upon deposition, airborne microorganisms affect phytoplankton/bacterial populations in surface seawater [7,12–14], and contribute to bacterial production [13] and N_2 fixation [7,14], impacting both the carbon (C) and the nitrogen (N) cycles.

The impact of airborne microbes may be particularly important in low-nutrient low-chlorophyll (LNLC) regions, which make up 60% of the global oceans [15], particularly where or when aerosol deposition rates are high. The Gulf of Aqaba (GOA) in the northern Red Sea (NRS) is a LNLC region with high atmospheric deposition due to its proximity to the Arabian, Sahel, Negev, and Sahara deserts. This proximity leads to high annual rates of dust deposition (50–500 g m^{-2}) in the NRS, with average dust loads reaching ~40 µg m^{-3} and ~700 µg m^{-3} during normal non-storm conditions and single dust storm events, respectively [16–18]. Previous studies showed that dust deposition in the NRS surface waters may alter chlorophyll *a* concentration, especially during the stratified most oligotrophic conditions in summer [17].

Thus far, observed changes in phytoplankton abundance (*Prochlorococcus, Synechococcus*, and picoeukaryotes) following aerosol deposition events or in simulated deposition experiments, have been solely attributed to the chemical

constituents of aerosols (i.e., nutrients) [19], while biotic constituents have been typically ignored [7]. This has also been the case for the NRS [20], despite reports that diverse arrays of microorganisms are present in aerosols in neighboring systems [13,21,22], including the NRS [23].

Currently, the impact of airborne microbes on native phytoplankton and bacterial populations and putative antagonistic or synergistic relationships that may occur are still poorly assessed. In this study, we investigated the role of dust-associated airborne microbes on primary and bacterial production using the NRS as a model ecosystem. To this end, we conducted mesocosm experiments where "live dust" (containing potentially viable airborne microbes and nutrients) or "UV-treated dust" (contributing only leached chemical constituents) were added to surface seawater collected from the NRS. Microbial abundances and both primary and bacterial production were measured daily over 72 h. Metagenomics was used to assess how the surface water microbial community changed after the dust amendments and differences between live and "UV-treated" dust additions assessed.

2.2. Methods

2.2.1. Dust Collection

Dust was collected at the NRS (29°28′ N, 34°55′ E) on 18 May 2017, during a large storm event that originated from the Sahara Desert [7]. Dust particles were collected on pre-cleaned glass plates and kept frozen (which may have impacted viability of some of the organisms) until the experiment in July 2017. Prior to the

experiment a subsample of the dust was placed under UV light for 48 h to kill the microorganisms associated with it (hereafter referred to as “UV-treated dust”). It has been shown that >95% of airborne microbes are inactivated by this UV treatment [7]. The remaining dust samples were left as is (hereafter referred to as “live dust”). Thus, live dust contributed nutrients, trace metals, and airborne microorganisms to the microcosms, whereas the UV-treated dust contributed only nutrients and trace metals.

2.2.2. Experimental Setup

In order to assess the specific contribution/impact of viable airborne microorganisms following dust deposition events in the NRS, surface seawater (from ~10 m depth) from the NRS was homogeneously distributed into nine polycarbonate mesocosm bags (each 300 L) on 9 July 2017. The mesocosms were submerged in a shaded pool with circulating seawater to maintain ambient temperatures (25–28 °C) and low light intensities (80–100 μmol quanta m^{-2} s^{-1} during midday, LI-COR PAR sensor) (Figure 1). The mesocosms were amended with the following treatments, in triplicate: (1) seawater with the addition of 0.8 mg L^{-1} of dust (live dust), (2) seawater with the addition of 0.8 mg L^{-1} of UV-treated dust, and finally (3) unamended seawater as a control (seawater with no dust added to simulate normal non-dust storm conditions). The amount of dust added (0.8 mg L^{-1}) was within the range of natural atmospheric deposition to the upper mixed layer of the NRS (~15 m) during intense dust storm events [17,24,25].The bags were mixed and subsampled before amendments were added at 6, 24, 44, and 72 h post dust additions as described below. We note that

what we refer to as dust includes not only mineral dust but also other aerosol constituents that were deposited along with the mineral dust during the storm.

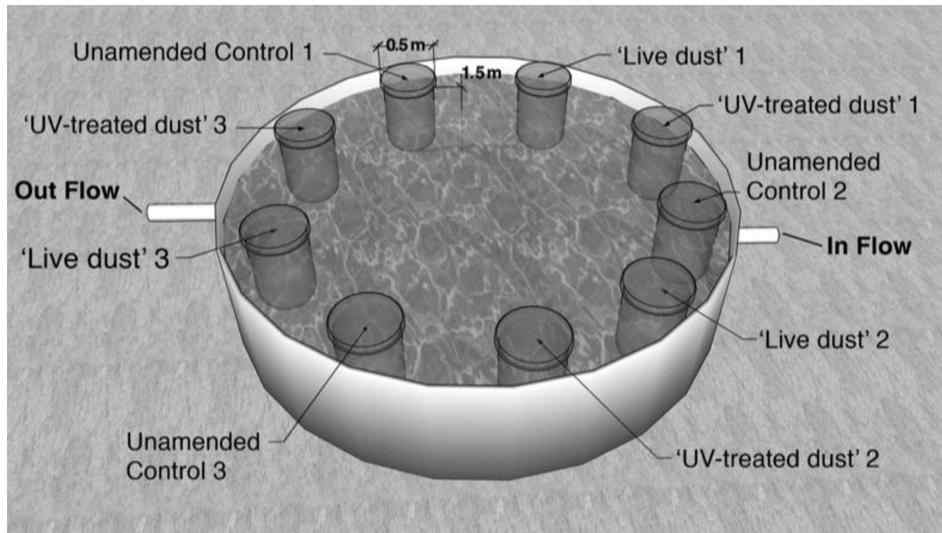

Figure 2.1. An illustration of the experimental mesocosm used in this study. Treatments included unamended controls, live dust and UV-treated dust run in triplicate 300 L transparent bags.

2.2.3. Bacterial Production (BP) and Primary Production (PP)

To quantify the impacts of airborne microbes on heterotrophic production, BP was measured using the (4,5-3H)-leucine incorporation method [26]. Briefly, seawater samples collected daily from the mesocosms (1.7 mL) were amended with 10 nmol of leucine L^{-1} (Perkin Elmer, specific activity 156 Ci $mmol^{-1}$) and incubated for 4–6 h in the dark. Incubations were stopped by the addition of 100 µL of cold 100% trichloroacetic acid (TCA). Control samples containing seawater, radioisotope, and TCA added immediately upon collection were also run daily. At the lab, the samples were microcentrifuged twice with TCA 5% and 1 mL of scintillation cocktail (Ultima-Gold) was added to each vial. Disintegrations per minute (DPM) were measured using

a TRI-CARB 2100 TR (Packard) liquid counter. A conversion factor of 1.5 kg C mol^{-1} per mole leucine was used [27].

To assess the impacts of airborne microbes on autotrophic production, the PP was measured following the ^{14}C incorporation method [28]. Briefly, water samples were analyzed in triplicate with dark and zero time controls. The samples (50 mL) were collected at 8:00 a.m. into transparent polycarbonate bottles (Nalgene) and amended with 5 µCi of $NaH^{14}CO_3$ (Perkin Elmer). The bottles were incubated for 4–6 h in the same pool where the mesocosms were placed. The incubations were terminated by filtering the spiked seawater onto GF/F filters under low pressure (<50 mmHg). Excess ^{14}C-bicarbonate was removed from the filters by acidification with HCl (32%) overnight. After adding 5 mL of scintillation cocktail (Ultima-Gold) to each vial, the radioactivity was measured using a TRI-CARB 2100 TR (Packard) liquid counter.

2.2.4. Chlorophyll-a (Chl-a)

To measure Chl-*a*, a proxy for total phytoplankton biomass, subsamples of seawater (500 mL) collected from the different mesocosms were passed through a Whatman GF/F filter, and 90% acetone solution was used for overnight extraction. The Chl-*a* concentrations were quantified using the non- acidification method [29], using a Trigoly fluorimeter equipped with 436 nm excitation and 680 nm emission filters.

2.2.5. Picophytoplankton and Heterotrophic Bacterial Abundance

To quantify the abundance of picophytoplankton and heterotrophic bacteria, seawater subsamples (1.7 mL) were collected from the different mesocosm bags, fixed

with 50% glutaraldehyde (0.15% final concentration, Sigma G7651), incubated for 10 min at room temperature, snap frozen in liquid nitrogen, and stored in −80 °C until analyses within a few weeks. Cell counts were performed by flow cytometry (Attune, Applied Biosystems) equipped with a syringe-based fluidic system and 488 nm and 405 nm lasers. Cyanobacteria (*Synechococcus* and *Prochlorococcus*) and picoeukaryotes were detected based on the orange fluorescence of phycoerythrin (585 nm) and the red fluorescence of Chl-*a* (630 nm), side scattered and forward scattered at a flow rate of 100 μl min^{-1}. Heterotrophic bacterial cells were first stained with a SYTO9 solution for 10 min in the dark and then run at a low flow rate of 25 μL min^{-1} using a discrimination threshold of green fluorescence (520 nm). One μm beads (Polysciences) were used as internal reference.

2.2.6. β-Glucosidase (β-Glu), Aminopeptidase (AMA), and Alkaline Phosphatase (APA) Activity

To determine the rate of polysaccharide degradation by bacteria, *β-Glu* activity was determined by the 4-methylumbelliferyl-β-D-glucopyranoside (Sigma M3633) method [30], and AMA was determined by the L-Leu-7-amido-4-methyl-coumarin method. Substrate was added in triplicate to 1 mL water samples (final concentration of 50 μM) and incubated in the dark at an ambient temperature for 24 h. To assess the rate of scavenging of organic matter due to phosphate limitation, the APA was determined by the 4-methylumbeliferyl phosphate (MUF-P: Sigma M8168) method [31]. Substrate was added in triplicate to 1 mL water samples (final concentration of 50 μM) and incubated in the dark at an ambient temperature for 24 h. The increase in

fluorescence of 4-methylumbelliferone (MUF) was measured at 365 nm excitation, 455 nm emissions (GloMax®-Multi Detection System E9032) and calibrated against a MUF standard (Sigma M1508).

2.2.7. DNA Extraction, Library Preperation, and Sequencing

To assess the diversity of microorganisms, seawater (1 L) from each mesocosm was filtered onto 0.2 μm polycarbonate filters. Total DNA was extracted from the filters using the phenol chloroform method [32]. Total DNA was also extracted from the aerosols collected using the same method. Polymerase chain reaction (PCR) using primers 515 (forward) and 806 (reverse) for 16S rRNA and primers EUK7F (forward) and EUK570R (reverse) for 18S rRNA, with barcodes on the forward primer, were carried out using the HotStarTaq Plus Master Mix Kit (Qiagen, Valencia, CA, USA). The samples were pooled together in equal proportions (based on their MW and DNA concentrations), purified using calibrated Ampure XP beads, and used to prepare libraries using a Nextera DNA Sample Preparation Kit (Illumina). Libraries were loaded to a 600 Cycles v3 Reagent cartridge (Illumina) and sequenced by illumina MiSeq.

2.2.8. Bioinformatics

Samples were processed using the open-source Quantitative Insights into Microbial Ecology 2 (QIIME 2) pipeline [33]. Sequences were demultiplexed and barcodes were trimmed. Data were denoised using DADA2 [34], sequences were clustered into amplicon sequence variants (ASVs) which can be thought of as 100% operational

taxonomic units (OTUs). Taxonomic classifier was trained [35] using Greengenes [36] for 16S and Silva [37] for 18S. Taxonomies were assigned using the naive Bayes method [38].

2.2.9. Statistical Analysis

The data in figures and tables are means and standard deviations (n = 3). Differences between treatments were tested using analysis of variance (ANOVA) and Tukey's post-hoc testing, and a p-value of 0.05 was used to determine significance unless noted otherwise. All tests were performed using R.

2.3. Results

The initial properties of the NRS surface waters used in the experiment (i.e., control mesocosms) are as shown in Rahav et al. [7]. Briefly, the surface water of the NRS exhibited oligotrophic characteristics with low micro- and macronutrients levels that were representative of summer conditions in the NRS: NO_3 + NO_2 (140 ± 13 nM), PO_4 (8 ± 1 nM), DOC (74 ± 1 µM), Fe (8.5 ± 1.8 nM), Zn (8.7 ± 2.1 nM) and Cu (1.4 ± 0.9 nM) [39,40]. Additionally, bacterial abundance ($3.5 \times 10^5 \pm 15 \times 10^4$ cell/mL), bacterial production (1.41 ± 0.08 µg C L^{-1} h^{-1}), primary production (0.60 ± 0.01 µg C L^{-1} h^{-1}), ß-Gl (1.42 ± 0.07 nM L^{-1} h^{-1}), APA (5.58 ± 0.17 nM L^{-1} h^{-1}), AMA (2.60 ± 0.09 nM L^{-1} h^{-1}), Chl-*a* (0.28 ± 0.01 µg/L), and *Prochlorococcus* ($1.49 \times 10^4 \pm 179$ cell/mL), *Synechococcus* ($5.14 \times 10^4 \pm 1.04 \times 10^4$ cell/mL), picoeukaryote ($1.58 \times 10^3 \pm 118$ cell/mL) abundances in the surface water of the NRS were determined (Supplementary Table S1). Leached micro/macronutrient concentrations added by the live or UV-

treated dust additions to the mesocosms were similar, representing: ~48 nM $NO_3 + NO_2$ (+34% of the ambient levels) and ~2.4 nM PO_4 (+30%), 165 ± 2 nM DOC (+0.22%), 3.3 nM Fe (+39%), ~7 nM Zn (+77%), and <1 nM Cu (+28%) (Supplementary Table S2).

Table 2.1. The net response triggered by airborne microbes in the northern Red Sea (NRS) seawater 24–48 h post addition. Values were calculated as the difference between the "live dust" and "UV-treated dust". Statistically significant differences are highlighted in bold ($p < 0.05$).

Variable	T20 h	T48 h
Chl-*a* (μg L^{-1})	−0.13	−0.06
Prochlorococcus (Cells mL^{-1})	−2140	−1920
Synechococcus (Cells mL^{-1})	−7300	**−9100**
Picoeukaryotes (Cells mL^{-1})	−120	−10
Heterotrophic bacteria (Cells mL^{-1})	−82,000	−3000
PP (μg C L^{-1} h^{-1})	−0.08	**−0.52**
BP (μg C L^{-1} h^{-1})	−0.32	**0.83**
β-Glu (nmol L^{-1} h^{-1})	**1.49**	**1.24**
APA (nmol L^{-1} h^{-1})	0.20	0.46
AMA (nmol L^{-1} h^{-1})	0.48	0.69

Although we reported results for all time points, we only included results from 20 h and 44 h after dust additions, and not 72 h, in our statistical analysis. These time intervals were selected because this was when the maximum differences in parameters between treatments were observed. Moreover, many of the parameters began to decrease after 44 h (including in the control treatments), suggesting that the changes observed were more likely due to "bottle effects" than to the changes in response to nutrient or microbial additions.

2.3.1. Changes in Phytoplankton and Bacterial Abundance following Dust Additions

Chl-*a* was measured as a proxy for total phytoplankton biomass (Figure 2A). For all treatments Chl-*a* concentrations increased slightly 20 h after amendments, and then

steadily decreased throughout the experiment (Figure 2A). Overall, Chl-*a* concentrations were significantly higher in treatments amended with live dust and UV-treated dust as compared with the control 20–44 h after amendment (Table 1, Figure 2A). However, there were no significant differences between Chl-*a* concentrations of the live dust and UV-treated dust treatments.

Prochlorococcus (Figure 2B), *Synechococcus* (Figure 2C) and picoeukaryotes (Figure 2D) dominate autotrophic communities in the NRS during the summer time [41]. All three autotrophs' abundances were different in the dust amendments as compared with the control treatments (Figure 2B–D). *Prochlorococcus* abundances decreased dramatically immediately after amendment in both dust treatments, while in the control we saw a decrease at only 20 h after amendment (Figure 2B). There was a slight increase in all treatments at 44 h, followed by another decrease. *Prochlorococcus* abundances were significantly lower in the live dust treatments (mean 3.7×10^3 cells/mL) and UV-treated dust (mean 5.8×10^3 cells/mL) than in the control treatments (mean 9.0×10^3 cells/mL) at 20 h post addition (Figure 2B, Supplementary Table S3). *Prochlorococcus* abundance in the dust treatments remained significantly lower than the control throughout the remainder of the experiment (Figure 2B), with no significant differences between live dust and UV-treated dust at both 20 h and 44 h after amendment. *Synechococcus* abundances initially decreased in all treatments and increased again at 20 h (Figure 2C). *Synechococcus* abundances in the dust treatments decreased again at 44 h after addition and were significantly different between the control (mean 6.62×10^4 cells/mL), live dust (mean 4.73×10^4 cells/mL), and UV-

treated dust (mean 5.64×10^4 cells/mL), treatments (Figure 2C, Supplementary Table S3). *Synechococcus* abundance was significantly higher in the control than in the live dust (28%) and UV-treated dust (14%) (p-value < 0.05) treatments, and live dust treatments had significantly lower *Synechococcus* abundance (-9.0×10^3 cells/mL) than the UV-treated dust treatments (Table 1, Supplementary Table S4). Picoeukaryote abundance was significantly increased in the live dust at 20 h and at 44 h (mean 4.4×10^3 cells/mL, mean 5.2×10^3 cells/mL, respectively) and UV-treated dust (mean 4.6×10^3 cells/mL, 5.2×10^3 cells/mL, respectively) treatments than in the control (mean 1.9×10^3 cells/mL, mean 1.9×10^3 cells/mL, respectively) (Figure 2D, Supplementary Table S3), after which abundances declined (Figure 2D) paralleling the dynamic of total *Chl-a* (Figure 2A). There was no significant difference in picoeukaryote abundance between the live dust and UV-treated dust (Supplementary Table S4).

Heterotrophic bacterial abundance (BA) 20 h post-amendment in both dust treatments increased up to ~150% relative to control levels, after which BA remained constant (Figure 2E). The BA in the control remained relatively stable throughout the experiment (Figure 2E). There was a significant difference (p-value < 0.05) in heterotrophic bacterial abundance between the control (mean 3.68×10^5 cell/mL) and the live dust (mean 9.09×10^5 cell/mL) and UV-treated dust (mean 9.91×10^5 cell/mL) treatments at 20 h, and between the control (mean 3.96×10^5 cell/mL) and the live dust (mean 9.13×10^5 cell/mL), and UV-treated dust (mean 9.17×10^5 cell/mL) treatments at 44 h (Figure 2E, Supplementary Tables S3 and S4). There were no significant differences in BA between the live dust and UV-treated dust (Supplemental Table S4).

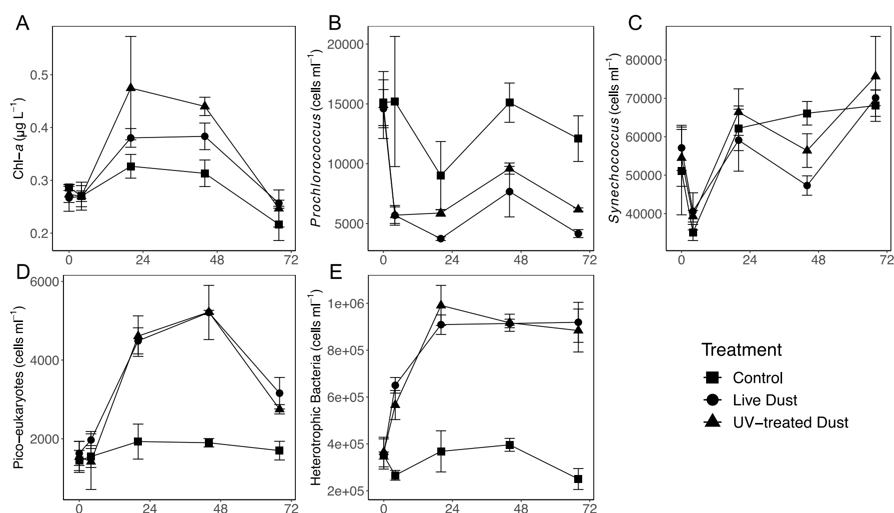

Figure 2.2. Temporal variability in Chl-*a* (**A**), *Prochlorococcus* (**B**), *Synechococcus* (**C**), picoeukaryotes (**D**), and heterotrophic bacteria (**E**) following 0.8 mg L^{-1} of "live dust" (circle), "UV-treated dust" (triangle) or unamended controls (square). Data shown are the average ± SD (n = 3).

2.3.2. Changes in Autotrophic and Heterotrophic Production following Dust Additions

Rates of primary production (PP) in all the treatments were relatively constant throughout the experiment with small differences between dust and control treatments, aside from 44 h after amendment (Figure 3A). The PP in live dust (20 h mean 0.66 µg C L^{-1} h^{-1}, 44 h mean 0.74 µg C L^{-1} h^{-1}), and UV-treated dust (20 h mean 0.74 µg C L^{-1} h^{-1}, 44 h mean 1.25 µg C L^{-1} h^{-1}) treatments were significantly higher than the control (20 h mean 0.58 µg C L^{-1} h^{-1}, 44 h mean 0.60 µg C L^{-1} h^{-1}) at 20 h and 44 h (Figure 3A, Supplementary Table S3). At 44 h, PP rates were significantly lower in the live dust treatments than in UV-treated dust treatments, with a net difference of 0.52 µg C L^{-1} h^{-1} (Table 1, Supplementary Table S4).

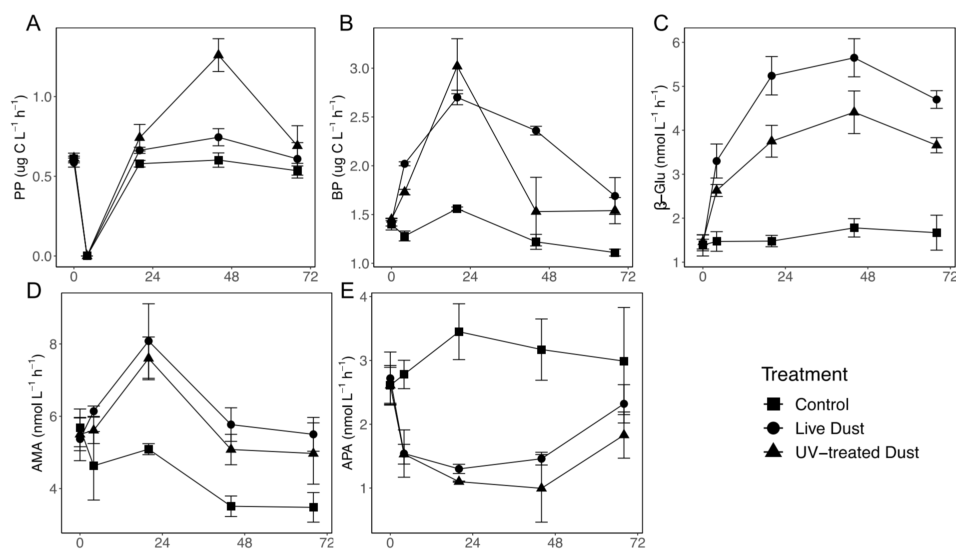

Figure 2.3. Temporal variability in primary production (PP) (**A**), bacterial production (BP) (**B**), beta-glucosidase (β-Glu) (**C**), leu-aminopeptidase (AMA) (**D**), and alkaline phosphatase activity (APA) (**E**) following 0.8 mg L^{-1} of "live dust" (circle), "UV-treated dust" (triangle) or unamended controls (square). Data shown are the average ± SD (n = 3).

Rates of heterotrophic bacterial production (BP) increased in the live dust (mean 2.70 μg C L^{-1} h^{-1}) and UV-treated dust (mean 3.02 μg C L^{-1} h^{-1}), treatments 20 h after amendment, and were significantly higher than rates of BP in the control (mean 1.56 μg C L^{-1} h^{-1}) (Figure 3B, Supplementary Table S3), respectively. At 44 h after amendment, BP rates decreased in the UV-treated dust treatments (mean 1.53 μg C L^{-1} h^{-1}) drastically while the live dust (mean 2.36 μg C L^{-1} h^{-1}) remained relatively stable (Figure 3B). Live dust treatments with airborne microbes had significantly higher rates of BP than both control and UV-treated dust treatments (Supplementary Table S4). The net BP rates of airborne microbes (difference between average BP rates in live and UV-treated treatments) was 0.83 μg C L^{-1} h^{-1} (Table 1).

Extracellular enzymatic activity rates were used as additional measures of the activity of different groups of organisms. Beta-glucosidase (*β-Glu*) (Figure 3C) and

leu-aminopeptidase (AMA) (Figure 3D) activities were used to measure the extracellular enzymatic activity of heterotrophic prokaryotes, whereas alkaline phosphatase activity (APA) (Figure 3E) rates were used to measure extracellular enzymatic activity of algae.

While *β-Glu* activity rates of the control remained relatively constant throughout the experiment (20 h mean 1.48 nM L^{-1} h^{-1}, 44 h mean 1.78 nM L^{-1} h^{-1}), *β-Glu* activity rates of the live dust (20 h mean 5.24 nM L^{-1} h^{-1}, 44 h mean 5.66 nM L^{-1} h^{-1}) and UV-treated dust (20 h mean 3.75 nM L^{-1} h^{-1}, 44 h mean 4.41 nM L^{-1} h^{-1}) treatments increased significantly 20 h and 44 h after amendments (Figure 3C). The ß-Gl activity rates were significantly higher in live dust treatments than UV-treated dust treatments (Supplementary Table S4), with airborne microbes contributing up to 1.24 nM L^{-1} h^{-1} (Table 1). The AMA rates in the live dust'(mean 8.08 nM L^{-1} h^{-1}) and UV-treated dust (mean 7.59 nM L^{-1} h^{-1}) treatments increased 20 h after amendment, whereas AMA rates in the control decreased (mean 5.09 nM L^{-1} h^{-1}) (Figure 3D, Supplementary Table S4). At both timepoints, both UV-treated dust and live dust treatments had higher rates of AMA (64% and 45%, respectively at 44 h) than the control (p-value < 0.05), but no significant differences between the live and UV-treated treatments were observed (Figure 3D, Supplementary Table S4).

The APA rates decreased for both live dust (mean 1.3 nM L^{-1} h^{-1}) and UV-treated dust (mean 1.097 nM L^{-1} h^{-1}) treatments while rates in the control (mean 3.45 nM L^{-1} h^{-1}) increased 20 h after amendment (Figure 3E). There were no significant differences

in the APA rates between the live and UV-treated dust throughout the experiment (Supplementary Table S4).

2.3.3. Dust-Associated Prokaryotes and Eukaryotes

The prokaryotic community in the dust sample collected and used in this experiment was comprised of bacteria from the Firmicutes (15%), Gemmatimonadetes (15%), Actinobacteria (13%) and Bacteroidetes (12%) phyla (Figure 4A). A large portion of prokaryotes (30%) were bacteria phyla that individually made up less than 1% of the relative abundance (Figure 4A). The eukaryotic community in the aerosols was dominated by Dikarya (55%), a subkingdom of Fungi containing the Ascomycota and Basidiomycota phyla, and Phragmoplastophyta (29%), a subclade of Charophyta (Figure 4B). All the organisms in the Phragmoplastophyta subclade were land plants (Supplementary Table S5). Of the eukaryotes in the dust, at least 8% were marine organisms (algae, protists) (Supplementary Table S5).

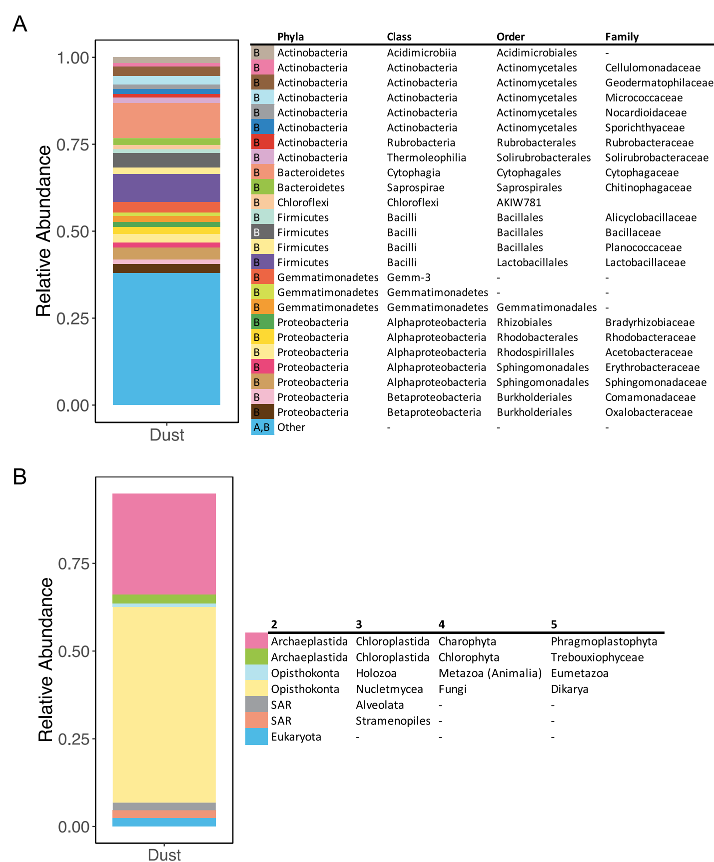

Figure 2.4. Relative abundance of prokaryotes (**A**) and eukaryotes (**B**) in the aerosols collected during the dust storm. The last row in both legends is the sum of all rare taxa (constituting <5% of relative abundance). In (A), A and B in the first column of the legend corresponds to archaea and bacteria, respectively. In (B), column names 2–5 represent taxonomic levels.

2.3.4. Changes in Prokaryotic and Eukaryotic Diversity following Dust Addition

The prokaryotic community in the NRS surface water was dominated by Proteobacteria constituting ~60% of taxa before the experiment, and ~70% to 75% during the experiment (Figure 5A). Alphaproteobacteria and Gammaproteobacteria were also abundant classes, making up 40–48% and 17–25% of the community, respectively (Figure 5A). Shannon's diversity index (H) and Faith's phylogenetic diversity (FPD) index were used to characterize the diversity of prokaryotes (Figure 6).

At both timepoints (20 h, 44 h), the diversity (H and FPD) was significantly different between the control and the dust treatments (Figure 6, Supplementary Figure S1). At 20 h, the prokaryotic diversity (H and FPD) of the control (mean H = 8.1, mean FPD = 21.2) was significantly higher than in both the live dust (mean H = 7.4, mean FPD = 13.0) and the UV-treated dust (mean H = 7.6, mean FPD = 14.4) treatments (Kruskal–Wallis test: H = 3.857, df = 2, $p < 0.05$) (Figure 6, Supplementary Figure S1). The opposite trend was seen at 44 h after amendment, where the diversity (H and FPD) of both live dust (mean H = 8.0, mean FPD = 19.9) and UV-treated dust (mean H = 7.9, mean FPD = 17.7) treatments was higher than the that of the control (mean H = 7.5, mean FPD = 14.8) (Kruskal–Wallis test: H = 3.857, df = 2, $p < 0.05$) (Figure 6, Supplementary Figure S1). Similarly, beta diversity (Bray–Curtis) showed no differences between the live dust and UV-treated dust treatments at 20 h and 44 h after amendment.

The eukaryotic community of the NRS was dominated by Alveolata (Dinoflagellata and Protalveolata) before the experiment (33%) and by Stramenopiles (Marine Stramenopiles and Ochrophyta) (33%) during the experiment (Figure 5B). The diversity of eukaryotes was also measured using Shannon's diversity (H) and Faith's phylogenetic diversity (FPD) indices (Figure 6). The control (mean H = 7.3, mean FPD = 38.2) had higher diversity than that in the live dust (mean H = 6.7) and UV-treated dust (mean H = 6.6 and mean FPD = 27.3) treatments 20 h after amendment (Kruskal–Wallis test: H = 3.857, df = 2, $p < 0.05$), with no differences between the live dust and UV-treated dust (Figure 6, Supplementary Figure S1). At 44 h after amendment, the

live dust treatments (mean H = 6.9, mean FPD = 29.7) had lower diversity than both the UV-treated dust (mean H = 7.3, mean FPD = 37.8) and the control (mean H = 7.3, mean FPD = 34.6) treatments (Figure 6). The beta diversity (Bray–Curtis) showed no differences between the live dust and UV-treated dust treatments at 20 h and 44 h after amendment.

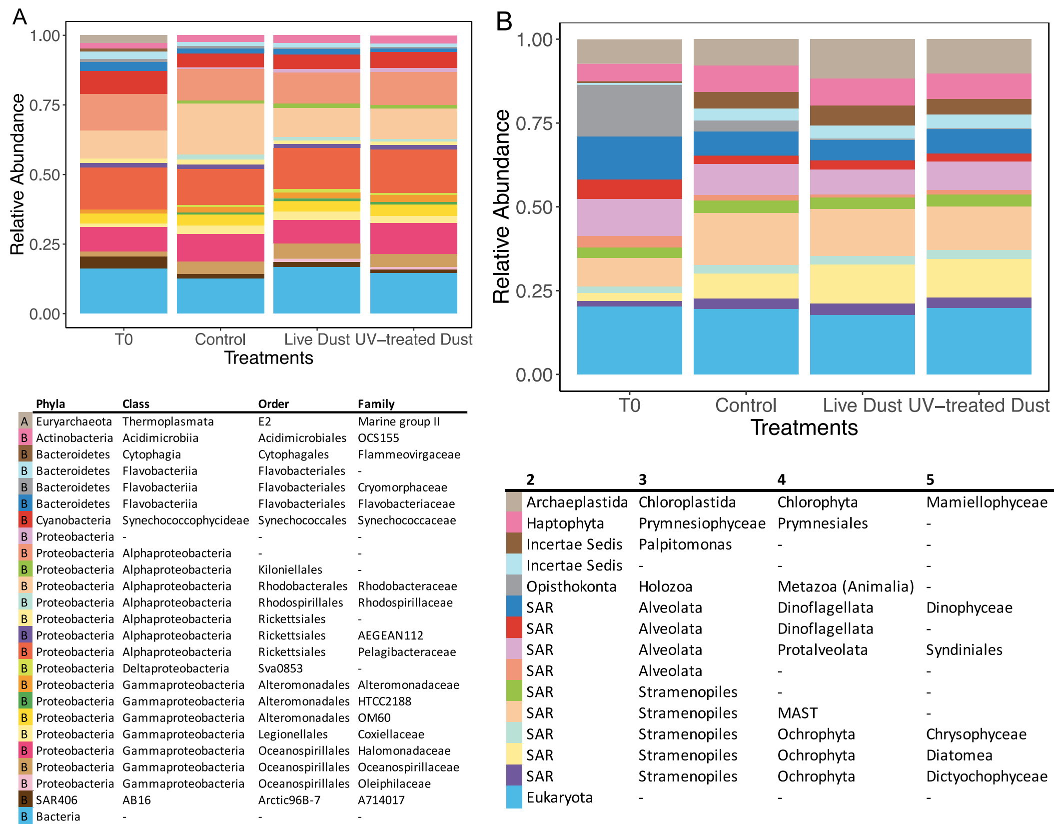

Figure 2.5. Relative abundance of prokaryotes (**A**) and eukaryotes (**B**) in the NRS surface water before amendment (T0) and 44 h after amendment for control, live dust, and UV-treated dust treatments. Data shown are the sum of triplicates. The last row in both legends is the sum of all rare taxa (constituting <5% of relative abundance). In (A), A and B in the first column of the legend corresponds to archaea and bacteria, respectively. In (B), column names 2–5 represent taxonomic levels.

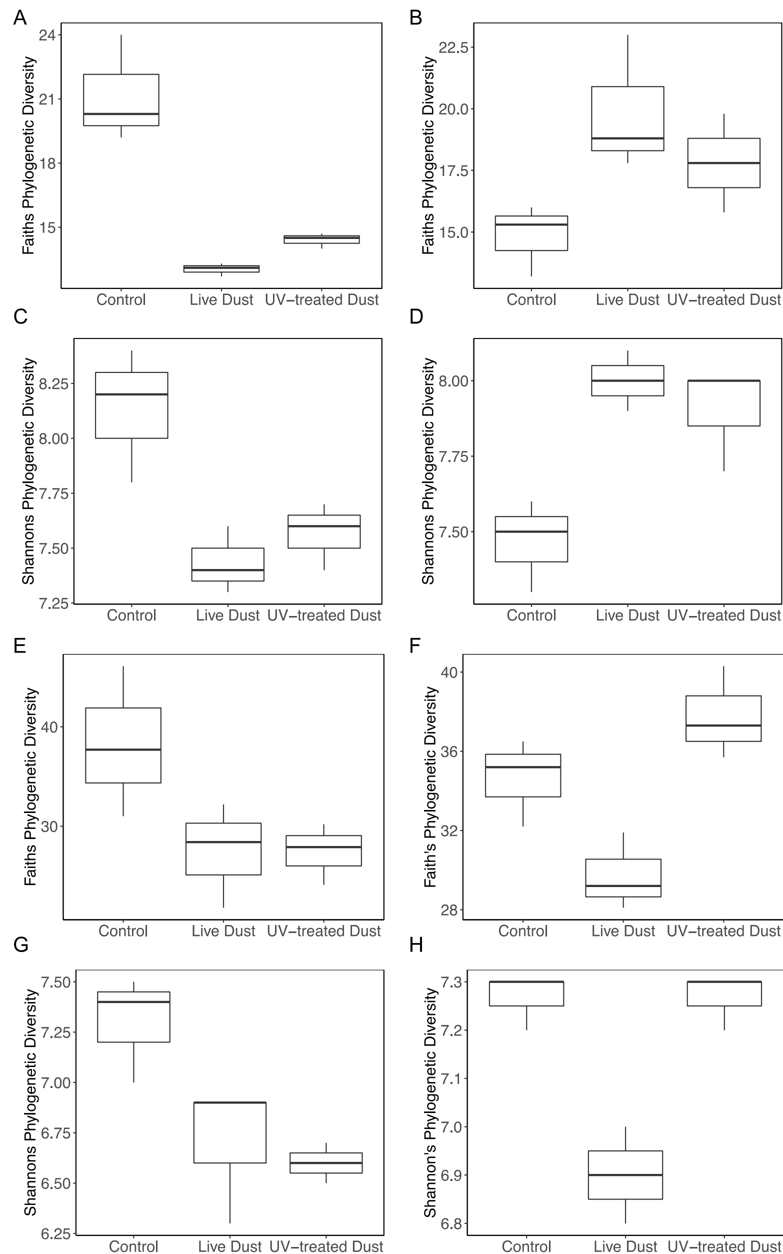

Figure 2.6. Diversity indices of prokaryotes, (**A**) Shannon's diversity indices at 20 h (**B**) Faith's phylogenetic diversity indices at 20 h, (**C**) Shannon's diversity indices at 44 h (**D**) Faith's phylogenetic diversity indices at 44 h, and diversity indices of eukaryotes, (**E**) Shannon's diversity indices at 20 h (**F**) Faith's phylogenetic diversity indices at 20 h, (**G**) Shannon's diversity indices at 44 h (**H**) Faith's phylogenetic diversity indices at 44 h, for control, live dust, and UV-treated dust treatments. The ends of the box are the upper and lower quartiles, so the box spans the interquartile range. The median for each treatment of three replicates is marked by a horizontal line inside the box. The whiskers are the two lines outside the box that extend to the highest and lowest observations.

2.4. Discussion

2.4.1. Impact of Airbone Microbes (or Dust Deposition) on Phytoplankton Biomass and Primary Production

The NRS receives high amounts of dust deposition every year, reaching dust loads of ~30 μg m^{-3} in normal non-storm conditions and ~700 μg m^{-3} during dust events [16–18]. Dust particles shelter and serve as a temporary habitat for microorganisms, protecting them from direct exposure to UV radiation [42,43]. Microorganisms survive long-range transport, and it has been reported that up to 25% of airborne microorganisms remain viable upon deposition [11,44] and subsequently thrive in novel marine ecosystems and impact native microbial populations and processes [13,14]. Low-nutrient, low-chlorophyll (LNLC) marine environments are strongly impacted by dust addition during high deposition events, because atmosphere is a crucial source of limiting nutrients and trace metals [19 and references therein]. However, the potential effects of airborne microorganisms delivered by atmospheric deposition in these areas has been often overlooked. Specifically, several studies in LNLC systems have shown an increase in primary production rates following aerosol addition and have solely attributed this outcome to the delivery of limiting nutrients (P, N, Fe) for photosynthesis [6,13,45-53]. Picophytoplankton, including cyanobacteria (*Prochlorococcus* and *Synechococcus*) and picoeukaryotes, account for a large portion of algal biomass and primary productivity in LNLC systems [41,54-57]. Two factors that drive picophytoplankton abundance in LNLC regions are (1) nutrient availability [58,59], and (2) population decline through viral infections and lysis [60]. Previous

studies have shown that atmospheric deposition affects picophytoplankton variably, both increasing abundance due to a supply of nutrients [45-47] and decreasing abundance due to a supply of toxic metals [20]. Comparing the effect of live dust potentially carrying live microorganisms and viruses (Figure 4) and UV-treated dust where microorganisms (and other biotic entities as viruses) were inactivated, we provide evidence that airborne microorganisms also have an impact on surface ecosystem.

Both live dust and UV-treated dust amendments led to an initial (0 to 44 h) increase of phytoplankton biomass as indicated by Chl-*a* (Figure 2A), mostly explained by the increase in picoeukaryotes (Figure 2D). This response likely resulted from the released nutrients from the dust particles that boosted cell growth (Supplementary Table S2). In contrast, cyanobacteria declined or showed small variations as compared with the control samples (Figure 2B,C). We note however that Chl-*a* and PP at 44 h were lower in the live dust samples, suggesting that living constituents present in the live dust negatively impacted phytoplankton growth and primary productivity rates (Figures 2A and 3A).

This effect could have resulted from the (1) introduction of competitors for the same food resources, for example heterotrophic bacteria [61,62] and/or (2) introduction of predatory and/or pathogenic entities, for example viruses [12,63]. Indeed, the dust used in the experiment contained bacteria as well as other marine organisms (Figure 4, Supplementary Table S5), which could compete with ambient phytoplankton for resources and may have served as vectors for viruses.

Viruses are abundant in desert soils, reaching concentrations of 2.2×10^3 to 1.1×10^7 virus-like particles per gram [64]. Upon aerosolization, viruses travel long distances via dust storms. The number of airborne viruses has been reported to increase by an order of magnitude during dust storms as compared with normal atmospheric conditions [65]. Dust storms also pick up marine viruses, which become aerosolized through wind-induced bubble bursting on ocean surface [12,63,66] while traveling over oceans. Some airborne viruses have been shown to infect phytoplankton in numbers large enough to terminate entire blooms [12]. Furthermore, there are viruses known to infect cyanobacteria that are very host-specific [67], potentially explaining why the three autotrophic populations which were measured here (picoeukaryotes, *Prochlorococcus* and *Synechococcus*) responded differently during the experiment.

2.4.2. Impact of Airborne Microbes (or Dust Deposition) on Heterotrophic Prokaryotes Production

Atmospheric dust deposition has also been shown to increase heterotrophic prokaryotes production rates in LNLC systems [5,14,46,48,68]. While these previous studies suggested that the input of nutrients from aerosols has increased in-situ surface water BP (i.e., that of resident heterotrophic bacteria in the marine surface layer), the differences in BP between the live dust and UV-treated dust treatments show that viable airborne microbes also rapidly contribute to increasing marine BP rates (by 50%, Figure 3B). Similar results, showing contributions of airborne microbes to BP, as well as to N_2 fixation, have been previously reported for southeastern Mediterranean waters [13,14]. The BP rates were previously tested in microcosm bioassay experiments where

aerosols collected during a dust storm were added to sterile southeastern Mediterranean water and showed that BP increased by fourfold [13], corresponding to 20–50% of the typical BP rates measured in the open and coastal southeastern Mediterranean, respectively [5,69]. Our study furthers their findings by showing that BP rates increase with the addition of airborne microbes in non-sterile water with resident microbial community from the Gulf of Aqaba in the NRS.

Our conclusion is also supported by the enzyme activity data (Figure 3C–E). Extracellular enzymes are synthesized by microorganisms to hydrolyze polymeric substances into bioavailable monomers [70]. Measuring rates of extracellular enzyme activity therefore provide insight into productivity of marine microbes. We show that AMA and β-Gl activities increased significantly with dust addition, while APA rates decreased significantly (Figure 3A–E). Synthesis of extracellular enzymes are dependent on nutrient availability (APA dependent on P, AMA and β-Gl dependent on organic carbon) and their activity rates change with the input of nutrients via atmospheric deposition [71,72]. The β-Gl activity is attributed mostly to heterotrophic bacteria [30,73], while AMA activity is attributed to heterotrophic bacteria as well as cyanobacteria, phytoplankton, and zooplankton [30,73-75] and its activities have been shown to increase after aerosol addition in a microcosm study in the Mediterranean Sea [72]. Specifically, β-Gl is utilized in hydrolysis of cellobiose found in polymers, such as cellulose and mucopolysaccharides, and can be related to Chl-a [76,77], whereas AMA is utilized in the decay of particulate matter composed of biotic and abiotic material [74,75]. APA, synthesized by phytoplankton [73], has also been shown to vary

in response to dust additions to the Mediterranean [71,72] and northern Red Sea [78] seawater during incubation experiments. Changes in enzymatic activity were observed in both the UV-treated dust and live dust treatments as compared with the control, showing that chemical components of atmospheric deposition are enhanced in-situ microbial activities, as previously suggested. However, we also measured differences in β-Gl activity rates between the UV-treated dust and live dust treatments, indicating that viable airborne microorganisms specifically contribute to increasing β-Gl activity rates (20–25%, Figure 3C). A significant difference between UV-treated dust and live dust treatments was not observed in the AMA activity rates (Figure 3D), which is also synthesized by heterotrophic bacteria. These results suggest that deposited airborne microbes preferentially synthesized β-Gl for hydrolysis of carbohydrates, and since the chemical constituents were the same in both treatments, the preferential use of this enzyme in the live dust treatment likely corresponds to synthesis by the microbial assemblage of the dust (Figure 4A).

Interestingly, although we saw differences in the BP between treatments, the heterotrophic bacteria abundance significantly increased with the addition of dust in both treatments relative to the control but was not different between the live dust and UV-treated dust treatments (Figure 2B). This difference in cell specific activity (bacterial production per bacterial abundance) was previously observed in a mesocosm study in the southeastern MS [13]. We attribute this increase solely to the chemical constituents of aerosols, which provide nutrients and organic C to increase heterotrophic bacteria abundance, in both treatments regardless of their origin

[5,14,46,48,68]. Alternatively, there may be a higher removal of heterotrophic bacteria in the live dust treatments by grazing or viral lysis, preventing an increase in abundance.

2.4.3. Microbial Population in the Dust and their Impact on Biodiversity

Most of the bacteria found in the dust (>1%) were not found in the seawater from the mesocosm experiments (>1%) at the family level, aside from organisms belonging to the family *Rhodobacteraceae* (Figures 4A and 5A). However, there was a large number of eukaryotes (Alveolates and Stramenopiles) present in the dust, as well as in all the seawater samples (Figures 4B and 5B). These organisms made up 8% of the dust (Figure 4B, Supplementary Table S5), and up to 52% of the seawater samples. These marine organisms likely become aerosolized from ocean surfaces through bubble bursting and sea spray [12,63,66].

We found similar taxonomic relative abundances in all treatments at 44 h after amendment (Figure 5), however this visual representation is not sensitive enough to detect differences in diversity. Therefore, to quantitatively assess community differences we calculated diversity of both prokaryotes and eukaryotes of treatments (live dust, UV-treated dust, and control) (Figure 6, Supplementary Figure S1) using alpha diversity metrics. Alpha diversity metrics are used quantitatively (Shannon's diversity index), qualitatively (Faith's phylogenetic diversity), and phylogenetically (Faith's phylogenetic diversity) to show how many unique taxa are present in a sample. Our experiment showed that eukaryote diversity (both measures) was different between live dust and UV-treated dust treatments at 44 h, with significantly lower diversity

when airborne microbes were present (Figure 6). The lower diversity in the live dust treatments indicate that the number of unique eukaryotes in the NRS surface water decreased with the addition of airborne microbes. The difference in diversity may be a result of competitive relationships between airborne microbes and eukaryotes in the NRS surface water. Alternatively, airborne viruses or fungi in our sample may have specifically infected certain marine eukaryote groups. However, the diversity of prokaryotes did not change between treatments, indicating that there was no antagonistic relationship between the bioaerosols and bacterial communities of the NRS. These processes need to be further studied.

2.5 Conclusions

Our results show that microbial diversity is altered by bioaerosols and that while the rates of primary productivity decline, the rates of bacterial production increase in response to the deposition of bioaerosols. Since our experiment lasted 72 h, with most changes occurring in the first 48 h, the observed effects of bioaerosols might be transient. Although our study was conducted in mesocosms and lasted only ~72 h, surface water with local microbial assemblages was used and the amount of aerosol added was representative of conditions during dust storm events. The results are consistent with previous reports indicating that atmospheric deposition is a crucial source of nutrients and trace metals in LNLC systems [2,3,79,80]. However, we also clearly show unique effects of airborne microorganisms impacting biogeochemical processes indicating that the impact of bioaerosols is also relevant, particularly in LNLC regions where dust storm events occur. Importantly, since dust deposition is

predicted to increase with climate change, the impact of airborne microorganisms found in our study may also increase. Additional studies that improve our understanding of how these geographically vast areas of ocean will be impacted by bioaerosols will be of great value.

2.6 Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1: Figure S1. Alpha diversity (Faith's phylogenetic or Shannon's diversity indices) for control, "live dust", "UV-treated dust" treatments; (**A**) Shannon's diversity index (DI) for prokaryotes at 20 h; (**B**) Faith's phylogenetic diversity (PD) for prokaryotes at 20 h; (**C**) Shannon's DI for prokaryotes at 44 h; (**D**) Faith's PD for prokaryotes at 44 h; (**E**) Shannon's DI for eukaryotes at 20 h; (**F**) Faith's PD for eukaryotes at 20 h.; Table S1. Chemical and biological properties of the NRS water used in the experiment (before amendments); Table S2. Nutrients and trace metals concentrations added from the aerosols to each mesocosm; Table S3. ANOVA test results between control, "UV-treated" and "live-dust' treatments at 20 h or 44 h; Table S4. Tukey post-hoc test results between "UV-treated" and control, "live-dust" and control and "live-dust" and "UV-treated' treatments at 20 h or 44 h; Table S5. Eukaryotes, relative abundances (abundance), and general information on taxa (details).

Author Contributions: Project administration and design A.P.; Writing—original draft, E.M.; Writing—review and editing, E.M., A.P., E.R., M.J.F., S.R., O.R., Y.G., C.S., and B.H.

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Conflicts of Interest: The authors declare no conflict of interest.

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Chapter Three

COLLECTION EFFICIENCY OF AIRBORNE MICROBES BY DIFFERENT INSTRUMENTS: A COMPARISON STUDY

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Abstract

Bioaerosols, including bacteria and fungi, are ubiquitous and have been shown to impact various organisms as well as biogeochemical cycles and human health. However, sample collection poses a challenge for aeromicrobiologists, and can determine the success of a study. Establishing a standard collection procedure for bioaerosol sampling could help advance the field. We tested the efficiency (number of organisms collected per unit time) of three sampling devices, a membrane filtration device, a liquid impinger, and a portable electrostatic precipitator bioaerosol collector. We compared the efficiency of these three devices for both culture-dependent studies, by enumerating colony forming units (CFUs), and culture-independent studies, by extracting and quantifying total DNA. Our results show that the electrostatic precipitator collected microorganisms significantly more efficiently than the membrane filtration and liquid impingement in both types of studies over the same time interval. This is due to the high flow rate of the device. This work is important and timely because aeromicrobiology is currently restricted by the time needed for sample collection due to evaporation, desiccation, freezing that increase the longer the sampling takes. In addition, fieldwork convenience and portability are an additional challenge. Using a sampler that can overcome these technical hurdles can accelerate the advancement of the field, and the lightweight, battery-powered, inexpensive, portable electrostatic precipitator bioaerosol collection device could address these limitations.

3.1 Introduction

Microorganisms can become aerosolized, transported and deposited by wind, and ~1-20% of these airborne microorganisms remain viable after deposition (Smith, 2013; Posfai et al. 2013; Prospero et al. 2005; Deguillaume et al. 2008; Womack et al. 2010; Polymenakou 2012). These airborne microbes, referred to as bioaerosols, can transmit diseases to new environments (Eames et al. 2009; Li et al. 2007; Roy et al. 2004; Yu et al. 2004), impacting humans, animals, and plants (Shinn et al. 2000; Hayes et al. 2001; Garrison et al. 2003; Weir-Bush et al. 2014; Griffin and Kellogg, 2004; Griffin et al. 2016). Recent studies have also demonstrated that airborne microbes deposited into the ocean can contribute to increases in marine bacterial production (Rahav et al. 2016) and N_2-fixation rates (Rahav et al. 2016; 2018), impacting nutrient cycles and possibly the biological carbon pump. Although the importance of studying airborne microbes is evident, environmental aeromicrobiology (that is the abundance and diversity of airborne microbes in open spaces) is still a relatively unexplored field. However, recent advances in molecular biology, specifically the availability of affordable and rapid genetic sequencing and bioinformatics, have advanced the field (Behzad et al. 2015). One of the limitations of the field is the identification and universal use of optimal sampling methods that provide good sensitivity and specificity for various types of analyses.

Many environmental aerobiology studies that have addressed multiple microbes (bacteria and fungi) or viruses have utilized different collection protocols, devices and

analytical assays (Gandolfi et al. 2013, Behzad et al. 2015), and this lack of standardization interferes with the ability to compare data between studies (Gandolfi et al. 2013, Behzad et al. 2015). This is especially true for studies that require quantification techniques such as quantitative polymerase chain reaction (qPCR), epifluorescence microscopy and flow cytometry (Gandolfi et al. 2013), but also applies to qualitative bioaerosol microbial diversity studies (Gandolfi et al. 2013). Until recently, most diversity studies on airborne microbes have used culture-based methods (Griffin et al. 2003; 2007; Prospero et al. 2005). These methods rely on fast collection rates, short enough to ensure cells will not be desiccated and hence remain viable. Although culture-based studies have been key to advancing microbiology, only 1-10% of total bacteria and fungi are culturable in the laboratory (Amann et al. 1995), and therefore, these methods shed light only on a small portion of the airborne microbial communities. Recently, there has been a shift to using next-generation sequencing for assessing airborne microbial diversity (Metzker, 2009, Rahav et al. 2016; Mazar et al. 2016; Gat et al. 2017; Mayol et al. 2017; and many more), which provides a more complete representation of the microbial communities (Sharpton, 2014) and has the potential to shed unprecedented light on bioaerosol diversity (Peccia et al. 2010). However, application of metagenomic sequencing techniques relies on high DNA yields of sufficient quality, which can be challenging due to the low biomass in most outdoor aerosol samples (between 10^4 or 10^6 microbes m^{-3}) (Lighthart et al. 1997), thereby requiring the collection of large quantities of air in relatively short periods.

Membrane filtration-based devices (MF) and liquid impingement (LI) devices are the most commonly used instruments by aeromicrobiologists (Fahlgren et al. 2011; Fields et al. 1974; Jensen et al. 1992; Kesavan et al. 2010; Griffin et al. 2001; Buttner et al. 1997), who study the microbial community of air in many environments including indoors, mountains, the ocean, and the lower atmosphere using small unmanned aircraft systems. Chen and Li [2005] used a MF sampler to test *Mycobacterium tuberculosis* levels in an indoor healthcare facility to develop a detection method using quantitative polymerase chain reaction (qPCR). In contrast, Angenent et al. [2005] used LI to detect and identify microorganisms in a hospital therapy pool. Tanaka et al. [2019] and Smith et al. [2013] both used MF-based instruments on mountains to determine high altitude airborne microbial communities. Griffin et al. [2010] used both MF and LI devices in the Mount Bachelor Observatory in Bend, Oregon, and compared the CFUs in samples collected by each instrument. In another high-elevation setting, Bowers et al. [2012] used MF to attain bacterial counts in order to study bacterial community shifts throughout the seasons. While investigating the annual variability of airborne microbes on the coast of the Baltic Sea, Fahlgren et al. [2010] used MF to quantify CFUs. Similarly, aeromicrobiologists who collect samples over the ocean typically install instruments on the upper deck of research vessels, and the samplers that are currently used include MF-based devices (Griffin et al., 2007; Xia et al., 2014; Mescioglu et al., 2019), impingers (Cho & Hwang, 2011) and, less commonly, cyclonic collectors (Mayol et al., 2017). Studies using both conventional (Kellogg et al., 2004; Prospero et al., 2005) and molecular methods (Rahav et al., 2016) investigating airborne microbes

during dust events have typically used MF systems. Cyclone-based collectors have also been developed for short-term sampling of aerosols to monitor environmental and occupational bioaerosol exposure (Tolchinsky et al., 2011). More recently, researchers have used remote-controlled small unmanned aircraft systems (sUAS) to collect airborne microorganisms from the lower atmosphere (Jimenez-Sanchez et al., 2018).

MF collection devices work by pumping air through a membrane filter composed of a chosen material and pore size. MF devices are low-cost, easy to build and operate, and are used widely in aerosol chemistry research (Aparicio-Gonzáles et al. 2012) as well as in aeromicrobiology (Prospero et al. 2005; Brodie et al. 2007; Griffin et al. 2007; Bowers et al. 2011; Jiang et al. 2015; and many more). MF systems used for aeromicrobiology are set to have airflow rates between 10-30 l min^{-1} that limit cell stress due to impaction (Fahlgren et al. 2011). Some of the disadvantages of MF include loss of cell viability with increased collection time due to desiccation (Griffin et al. 2010). It is convenient to use filters in culture-based studies by placing the filters with the samples, facing up, onto agar plates. The filters then act as a wick and bring the nutrients up to microorganisms collected onto the filter, allowing viable microbes to develop colonies on the filter. However, it is challenging to use filters in culture-independent studies because it is necessary, yet not trivial, to remove microorganisms from the filter before downstream processing to prevent the inhibitory materials of the filter from reducing assay efficiency (Despres et al. 2007).

LI devices work by pumping air through an inlet into liquid collection medium, and can have multiple compartments that separate particles based on size fractions. LI has a higher airflow rate than the MF, which reduces collection time, and has a lower likelihood of cell desiccation since the organisms are kept in liquid during sampling. It is also possible to use the sampled liquid in multiple assays by easily dividing the homogeneous collected material (Griffin et al. 2010). Since cells are already in liquid, the medium can be centrifuged to concentrate cells to a smaller volume and used directly in nucleotide extraction kits. However, LI devices are less convenient to use in the field since they are heavy, need to be autoclaved after each use, and are not recommended for long sampling periods due to evaporation (Grinshpun et al. 1996) or for sampling in high latitudes due to freezing of the liquid medium.

There are also volumetric air sampling devices, such as the Burkard sampler or one designed by Pastuszka et al. [2013], that impact aerosols directly onto agar plates instead of onto filters. These samplers have similar flow rates (10-30 l min^{-1}) to MF devices (Pastuszka et al. 2013), but likely increase cell stress and death due to direct contact with the agar (Stewart et al. 1995). Furthermore, these devices are used less commonly than MF-based devices in studies where information regarding the total airborne population is desired, but seem to work well for fungal spore collection (Ho et al. 2004; Wu et al. 2004).

A less commonly used sampler is an electrostatic precipitator (EP), which uses a high voltage electric charge to attract airborne particles to a grounded surface. Studies have used a variety of EP collection devices to collect airborne microbes (Grinshpun et al. 1996, Mainelis et al. 1999; 2002a; 2002b; Hogan et al. 2004; Dybwad et al. 2014; Mbareche et al. 2018). Specifically, an EP sampler developed by the United States Department of Agriculture is small, lightweight (0.9kg), inexpensive, portable, and battery-powered (Gast et al. 2004). The battery lifetime of the device is ~9 hours using standard 9 V batteries (500 mAh) and ~21 hours using 1200 mAh batteries, and the unit can be adapted to run using a 12 V source or an AC adapter. The USDA EP has a relatively high air flow rate (100 l min^{-1}) and can be used to collect airborne microbes directly onto agar media plates (Gast et al. 2004). The USDA EP can be used in the field during multiple consecutive sampling runs because the sampler can be disinfected by spraying the EP and switching the used agar plate with a new premade sterile agar plate after sterilization at the beginning of each run. This specific EP has been used in the detection of the pathogen *Salmonella enteritidis* in poultry house environments alongside an impaction device and a passive exposure collector (Gast et al. 2004). The EP was the most reliable of the devices tested in the *S. enteritidis* detection study (Gast et al. 2004). At present, however, the EP is designed to work only with agar plates, which works for culturing and but is not the best "substrate" for genetic material (DNA/RNA) extraction. Thus, the designer of the EP has suggested collecting samples onto a bare metal plate for culture-independent studies and washing off microbes with

a PSB solution for downstream processing. This EP has not yet been tested in a culture-independent study where DNA yield is quantified.

Aerobiology studies would be advanced by use of an aerosol sampling instrument that can provide a solution to the evaporation, desiccation, freezing, and fieldwork convenience problems, such as power source, size and weight of collector, and disinfection between runs, and that can be used for both culture-dependent and culture-independent studies. Importantly, the success of any instrument hinges on the efficiency of the collection (the number of organisms collected per unit time) and the representativeness of the collected assemblage. Here we compare EP, LI, and MF devices operating simultaneously in St. Petersburg, Florida, during normal atmospheric conditions to evaluate how they compare in the efficiency of collection for culture-dependent and culture-independent studies over the same time of collection.

3.2 Methods

3.2.1 Samplers and the Experimental Set-Up

A multi-stage LI (Burkard Manufacturing Co Ltd, United Kingdom) with three particle size fractions (> 10 μM, 10-4 μM, < 4 μM) was used in the experiment. A MF system that was assembled in-house (110 V vacuum pump, Fisher Scientific, PVC two-place-manifold, and housing) was used with pre-sterilized filter housings containing 47-mm-diameter, 0.2-μM-pore-size cellulose acetate filter membranes to collect samples (Fisher Scientific, Atlanta, GA). The EP used was manufactured by the United States

Department of Agriculture (Gast et al. 2004) with a reported hypothetical flow rate of 100.05 l min^{-1} (Gast et al. 2004), which was used in our calculations. The flow rates of the MF and LI samplers were measured before each sampling event and were 11.491 l min^{-1} and 0.9352 l min^{-1}, respectively.

The LI, MF, and EP samplers were tested outdoors at ground level during the daytime at the U.S. Geological Survey in St. Petersburg, Florida. The samplers were set next to one another, and metadata, including start and end time, temperature, humidity, and flow rates were collected for each run (Table 1). A particle counter (IQAir Particle Scan Pro) was set up alongside the samplers to assess levels of particulate matter in the air during the sampling runs. The particle counter was used to report six size fraction ranges (≥0.3 μM, ≥0.5 μM, ≥0.7 μM, ≥1.0 μM, ≥ 2.0 μM, ≥5.0 μM). The duration of sample collection for the culture-dependent and culture-independent experiments were approximately 1 to 2 hours and 2 hours, respectively. A total of 5 samples were collected for both the culture-dependent and culture-independent study sample sets over five days, and we did not include replicates within the same run because only a single device for each system was used.

3.2.2 Culture-dependent experiments

Tryptic soy agar (TSA) media was used to culture the microorganisms. Samples were collected directly onto agar plates with the EP. For the MF samples, filters were removed from the plastic holder and placed onto an agar plate facing up using sterile

forceps. The LI was autoclaved between runs and prepared with 7 mL of sterile 1x phosphate saline buffer (PSB) in each of the three compartments. After the run was completed, liquid from the impinger was pipetted into 15mL tubes and centrifuged to a pellet at 5,900 x g for 20 minutes. The liquid above the pellet was pipetted off until 1 mL remained. The pellet and remaining liquid were then mixed thoroughly by vortexing, and 200 µL was spread onto an agar plate (in triplicates). All the agar plates were incubated at 36º C, and CFUs were enumerated manually after ~36 hours. For the LI the averages of the triplicate CFU values were used in the analysis.

3.2.3 Culture-independent experiments

Membrane Filters

The membrane filters were kept in a -20 degrees freezer following collection and until processing (between 3 and 7 days). The filters were placed into autoclavable and sterilized 47mm filter holders and backflushed using 15 mL of sterile 1x PBS to remove collected microbes from the filter. The liquid was pelleted at 5,900 x g for 20 minutes, and excess liquid was pipetted off until 1 mL remained. The samples were vortexed, and 200 µL was used to extract DNA.

Liquid Impinger

The PSB solution containing bioaerosols was pipetted from each compartment into separate sterile tubes. The solution was reduced in volume by evaporation to ~5 mL. PSB solution (0-2 mL) was added to each sample to a final volume of 7 mL. The PSB solution with bioaerosols was then pelleted down at 5,900 x g for 20 minutes, and

excess liquid was pipetted off until 1 mL remained. The samples were vortexed to homogenize, and 200 μL was used for DNA extraction.

Electrostatic Precipitator

The EP was used in two ways. For samples collected by the instrument we refer to as EP for culture-independent studies, samples were collected onto a sterile metal plate without agar, rinsed with 10 mL of PSB, centrifuged to pellet (5,900 x g for 20 minutes), decanted to 1 mL. The pellet was vortexed with the remaining 1 mL solution, and 200 μL was used to extract DNA. For samples collected by the instrument we refer to as EP_A, we collected material onto a TSA agar plate (normal operation) and transferred the material into two DNA extraction tubes, which were later combined, using swabs.

DNA was extracted from all the samples using the Qiagen DNeasy PowerSoil Kit following the manufacturer's protocol until the last step, where instead of using the elution buffer, Qiagen AE was used to elute DNA. DNA was quantified using a Qubit Fluorometer with the Qubit dsDSNA HS Assay Kit and reported in ng μL^{-1}.

Statistical Analysis

All statistical tests were carried out using R. We did not process the data beforehand, except for normalizing the results to the volume of air pumped. It was not necessary to control for additionally measured co-factors because 1) there was no clear relationship

between the measured co-factors and the DNA yield (even after log transformation of data), and 2) while humidity and temperature had some effect on CFUs, the effects of the instrumentation was much stronger. Furthermore, the experimental design controlled for these co-factors because each sampler was run at the same time and location alongside the other samples, and therefore, they have the same range in temperature and humidity. CFU and DNA yield data were both non-parametric; thus, Kruskal-Wallis test was used to test differences between groups. Spearman's test was used to test for correlation between two variables.

3.3 Results

3.3.1 Particle Counts

The particle concentrations in the air varied throughout the study. The average abundances for the six size fractions and the total particle counts are reported for culture experiments and nucleotide experiments in Supplementary Figures 1 and 2, respectively. Most of the particles during the experiments were in the ≥0.3 µM size range, and therefore the number of total particles is primarily influenced by particles ≥0.3 µM. The total number of particles ranged from 2.3×10^4 to 6.83×10^4 per liter of air (median = 4.04×10^4) during the collection for the culture-dependent experiment. Run L and run A had the highest number of particles in all size fractions for the culture-dependent experiments. Total particles ranged between 2.35×10^4 and 7.40×10^4 per liter of air (median = 3.42×10^4) during collection for the culture-independent experiments, with the highest number of particles in run C and run U. Run U had the

highest number of particles in all size fractions, except for particles ≥0.3 μM, which were highest in number during run C.

3.3.2 Culture-dependent Experiments Comparison

The number of total colony forming units (CFUs) for samples collected with the LI ranged from 2 to 104 colonies (median = 8) (Table 2). The number of bacterial and fungal colonies were also counted separately (Table 2), and when the total number of CFUs was high (84 and 104), the bacteria made up 69% and 84% of the total CFUs, respectively. CFUs per m^{-3} of air for samples collected with the LI ranged from 3 to 186 (median = 12) (Table 2.)

CFUs in samples collected with the MF ranged from 1 to 80 CFUs (median = 5) (Table 2). The number of bacterial colonies ranged from 0 to 55, and there is no data on the fraction of bacteria and fungi in the sample with 80 total CFUs due to the similar appearance of many of the colonies (Table 2). CFUs per m^{-3} of air for samples collected with the MF ranged from 1 to 116 (median = 7) (Table 2).

The EP samples had the largest number of total CFUs grown, ranging from 22 to 929 CFUs (median = 77) (Table 2, Figure 1). Sample A_EP and L_EP had nine times more CFUs than the samples with the highest number of CFUs from the other samplers (LI sample L_L with 104 CFUs). For the samples with very high counts, it was not possible to accurately differentiate between the bacterial and fungal colonies because the

colonies appeared homogenous. CFUs per m-3 of air for the EP samples ranged from 3 to 160 (median = 13) (Table 2).

3.3.3 CFUs Relation to Particle Counts

There was a larger total of CFUs during experiments that corresponded with the highest particle counts (Figure 1). There was a significant positive correlation between the total CFUs collected by the LI and particle counts in the ≥0.5 μM, ≥0.7 μM, and ≥1.0 μM size fractions (Spearman's correlation: rho = 0.97 p = 0.0048). CFUs per m-3 of air collected with the LI were also correlated to particle counts in the ≥0.5 μM and ≥0.7 μM size fractions (Spearman's correlation: rho = 1.00, p = 0.01667). Total CFUs and CFUs per m-3 of air collected with the MF were significantly correlated to particle counts in the ≥2.0 μM size fractions (Spearman's correlation: rho = 1.00, p = 0.01667). The EP also had a larger total of CFUs and CFUs per m-3 of air when total particle counts and particles in the ≥2.0 μM size fraction were higher, but the correlation was not significant (Spearman's correlation: rho = 0.80, p = 0.080).

3.3.4 Culture-independent Experiments Comparison

The DNA concentration of samples collected using each of the instruments are listed in Table 3 and illustrated in Figure 2. Since the LI has three compartments, the highest concentration from the three was used for the analysis. DNA was not detectable (<0.50 ng/mL) by the Qubit dsDSNA HS Assay Kit in 6 out of 28 sample (4 = EP with metal plate, 1 = LI, and 1 = MF). The DNA yield was significantly different between the LI

(median = 0.02 μg/mL), MF (median = 0.021 μg/mL), EP with a metal plate (median = 0.05 ng/mL), and EP with an agar plate (median = 0.1355 μg/mL) (Kruskal-Wallis test: $H = 13.73$, $df = 3$, $p = 0.003296$). The EP with an agar plate yielded the highest concentration of DNA, significantly outperforming the EP with a metal plate ($p = 0.027$), the MF ($p = 0.026$), and the LI ($p=0.026$) (Figure 2) (Pairwise Mann-Whitney U test). Similarly, when nucleotide concentrations were normalized to the volume of air pumped, there was a significant difference between samplers (Kruskal-Wallis test: $H = 8.25$, $df = 3$, $p = 0.041$), but the difference was only significant between EP with a metal plate and EP with an agar plate (0.04). The nucleotide concentrations of samples did not significantly correlate to particle counts regardless of the collection instrument used. However, the highest concentrations of DNA throughout the experiment was collected during the run with the highest number of particles in the ≥0.5 μM, ≥0.7 μM, ≥1.0 μM, ≥2.0 μM, and ≥5.0 μM size fractions (Run U).

Discussion

Our results show that sampling for the same length of time resulted in a larger total of CFU's in samples collected by the USDA EP than the LI and MF devices, indicating that the USDA EP was more efficient when testing culture-dependent methods (Figure 1, Table 2). CFU's per m^{-3} of air were not significantly different between the instruments, and during two runs (L and V), the EP collected fewer CFU's per m^{-3} than the MF and LI. These results indicate the high flow rate of the EP results in an increase in total microbes collected and, hence CFUs recovered. Although the LI collected more

CFU's per m^{-3} of air during two sampling events, the sampling duration would have to be increased by ~2-20 fold to ultimately collect the same absolute number of CFU's as the EP (Table 2). These results indicate that all three sampling devices collect similar numbers of culturable organisms from a volume of air, but because the EP has a much higher airflow rate, more organisms are retrieved per unit time. This is an important quality because a sampler that can collect more airborne organisms over a shorter time will potentially allow the detection of rare pathogens that otherwise would be missed, and samples can be processed before quality degradation. Moreover, samplers have to be practical. It would take hours for the LI and MF samplers to achieve a similar sample yield (defined here as collection efficiency). While the total number of airborne microbes collected and cultured does not itself correlate to real-life health impacts, the increased chance to detect rare pathogens is relevant as early detection is important to curtail the spread of contagious disease. Thus, if MF and LI can be set to pump air at faster rates while not compromising the viability of airborne microbes, they as well can be used effectively. We suggest that additional tests with higher flow rates for these devices be carried out.

The USDA EP used with an agar plate yielded the highest concentrations of DNA (Figure 2, Table 3) in our experiment, indicating that it is more efficient and effective than the LI and MF devices Figure 2, Table 3). While the EP with a metal plate, LI and MF devices almost always yielded between undetectable and <0.1 μg/mL of DNA (5 out of 6 and 6 out of 6 runs, respectively), the EP with an agar plate consistently (5 out

of 6 sampling events) yielded >0.1 μg/mL of DNA (Table 3, Figure 2). Similar to the CFU results, the concentration of DNA per m^{-3} of air were not significantly different between the instruments, indicating that the high flow rate of the EP was key in its outperformance of other samplers (more air pumped hence more microbes collected). It would be very interesting to determine if the higher DNA yield corresponds to the detection of rare organisms that are missed with the other instruments.

It is interesting and important to note the difference between DNA yield when using a metal plate and an agar plate with the EP sampler. One explanation could be that some microbes grew on the agar plates during the collection time. However, although we used TSA agar in our EP collection device, the plates were processed immediately after sampling, fast enough to prevent substantial growth that could account for the observed differences. There were no visible CFU's on the agar at the end of sampling. Since only ~1% of microorganisms are culturable in the lab in optimal conditions, we can rule out that growth could have led to the high DNA yield we observed. Alternatively, we hypothesize that the adhesive nature of the agar is effective in trapping particles with associated microorganisms and preventing them from desiccating, whereas the metal plate does not have the same effect and in fact, particles may bounce off the plate. Additionally, we hypothesize that washing the metal plate with PSB did not recover as many microorganisms as swabbing the agar plates to obtain the DNA. It would be interesting to use liquid (similar to the LI system) instead of agar for a more direct comparison of the effect of trapping or bouncing of the different collection alternatives.

Bioaerosols are found in indoor (Tringe et al. 2008; Kembel et al. 2012; Rintala et al. 2008; Adams et al. 2014; Dunn et al. 2013) and outdoor environments (Kellogg and Griffin, 2006; Griffin et al. 2007; Katra et al. 2014; Rahav et al. 2016; Gat et al. 2017; Mayol et al. 2017), and may impact both human health (Kellogg et al. 2004; Sultan et al. 2005; Brodie et al. 2007; Oh et al. 2014; An et al. 2014) and natural ecosystems (Sharoni et al. 2015; Rahav et al. 2016, Rahav et al. 2018). Despite their importance, particularly given of future decreases in air quality and increasing desertification, there are no standardized methods of studying bioaerosols (Behzad et al. 2015). This makes conducting new aeromicrobiology studies difficult due to issues related to replicating, interpreting, and comparing existing studies (Behzad et al. 2015). Because the biomass of airborne organisms in aerosol samples is low, one of the most challenging aspects of aeromicrobiology studies is sample collection and establishing an efficient (i.e., reduction in time and complexity of operation) and effective (i.e., obtaining an accurate and representative assessment of organisms in the air) collection instrument would help advance the field.

Although previous studies have compared different collection instruments in parallel, this is the first to compare the EP recently manufactured by the USDA (Gast et al. 2004) and two more commonly used collection devices (LI and MF). We found higher yield using the USDA EP with agar plates for both culture-based (quantifying CFU's; Figure 1, Table 1,2) and culture-independent (quantifying DNA concentrations; Figure

2, Table 1,3) methods. The main driver for the increase in yield is the higher flow rates and effective capture efficiency generated by strong electrostatic attraction of the EP compared to the LI or FM samplers. The EP is also relatively lightweight, battery-powered, inexpensive, and portable. However, if other devices can achieve higher airflow rates without compromising trapping efficiency, they could also be as effective since the number of airborne microbes detected when normalizing to the volume of air pumped is similar for all instruments tested here.

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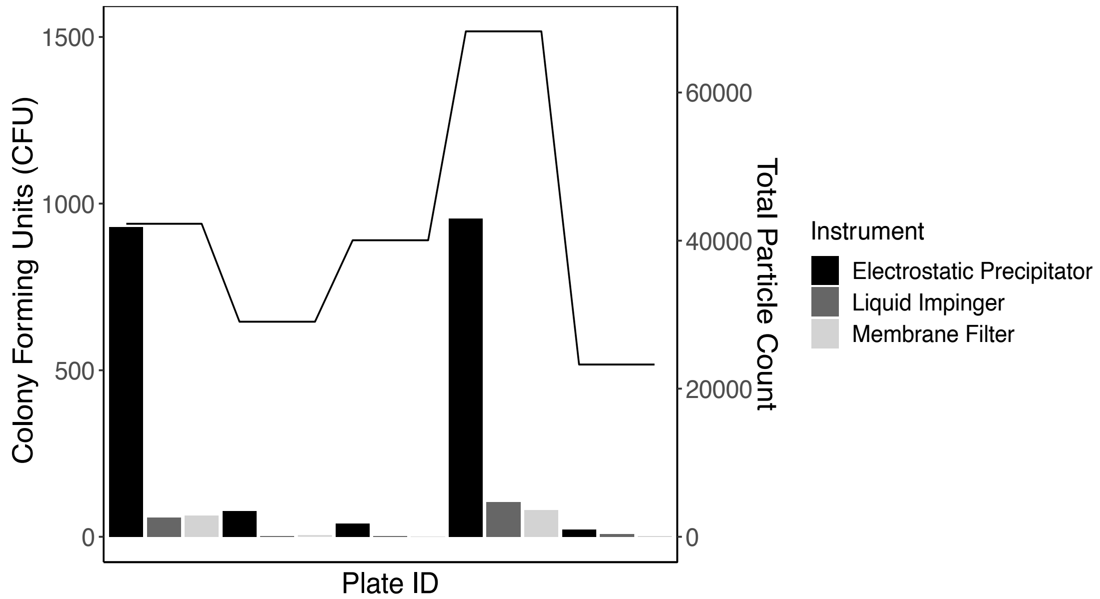

Figure 3-1. Colony-forming units (CFUs) shown as bars on the left y-axis and total particle counts shown as lines on the right y-axis, with each bar representing a plate. The colors correspond to the instrument used to collect samples onto each plate.

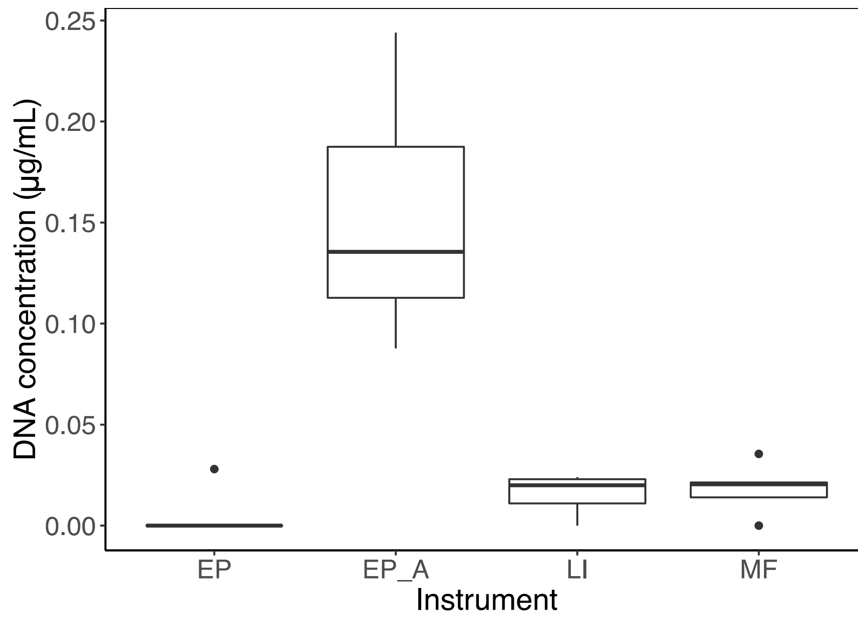

Figure 3-2. DNA yield shown with samples grouped corresponding to the instrument used to collect samples.

Study Type	Run ID	Sampler	Date	Start Time	Humidity	Temp (F)	End Time	Run Duration (h)	Run Time (min)	Flow Rate ($l\ min^{-1}$)	Volume of air (m^3)
Culture-dependent	A	MF	4-May-18	12:51	56.7	83.7	14:52	2:01	121	11.49	1.39
	A	LI	4-May-18	12:46	56.7	83.7	14:52	2:06	126	9.35	1.18
	A	EP	4-May-18	12:47	56.7	83.7	14:54	2:07	127	100.05	12.71
	D	MF	8-May-18	8:12	62.3	74.1	9:13	1:01	61	11.49	0.70
	D	LI	8-May-18	8:13	62.3	74.1	9:13	1:00	60	9.35	0.56
	D	EP	8-May-18	8:14	62.3	74.1	9:13	0:59	59	100.05	5.90
	J	MF	11-May-18	8:05	72	75.1	9:05	1:00	60	11.49	0.69
	J	LI	11-May-18	8:06	72	75.1	9:06	1:00	60	9.35	0.56
	J	EP	11-May-18	8:06	72	75.1	9:06	1:00	60	100.05	6.00
	L	MF	11-May-18	14:55	42.7	89.9	15:55	1:00	60	11.49	0.69
	L	LI	11-May-18	14:55	42.7	89.9	15:55	1:00	60	9.35	0.56
	L	EP	11-May-18	14:55	42.7	89.9	15:55	1:00	60	100.05	6.00
	V	MF	23-May-18	12:42	83	80.6	13:53	1:11	71	11.49	0.82
	V	LI	23-May-18	12:42	83	80.6	13:53	1:11	71	9.35	0.66
	V	EP	23-May-18	12:42	83	80.6	13:53	1:11	71	100.05	7.10
Culture-independent	C	MF	7-May-18	11:51	54.1	82.8	14:00	2:09	129	11.49	1.48
	C	LI	7-May-18	11:53	54.1	82.8	14:00	2:07	127	9.35	1.19
	C	EP	7-May-18	11:54	54.1	82.8	14:00	2:06	126	100.05	12.61
	E	MF	8-May-18	11:15	55.2	80.2	13:15	2:00	120	11.49	1.37
	E	LI	8-May-18	11:17	55.2	80.2	13:15	1:58	118	9.35	1.10

	E	EP	8-May-18	11:20	55.2	80.2	13:15	1:55	115	100.05	11.51
	I	MF	10-May-18	11:09	57.9	80.3	13:13	2:04	124	11.49	1.42
	I	LI	10-May-18	11:10	57.9	80.3	13:13	2:03	123	9.35	1.15
	I	EP	10-May-18	11:11	57.9	80.3	13:13	2:02	122	100.05	12.21
	K	MF	11-May-18	10:58	61.3	81.5	13:00	2:02	122	11.49	1.40
	K	LI	11-May-18	10:59	61.3	81.5	13:00	2:01	121	9.35	1.13
	K	EP	11-May-18	10:59	61.3	81.5	13:00	2:01	121	100.05	12.11
	M	MF	14-May-18	8:05	78	73	10:10	2:05	125	11.49	1.44
	M	LI	14-May-18	8:00	78	73	10:10	2:10	130	9.35	1.22
	M	EP	14-May-18	8:10	78	73	10:10	2:00	120	100.05	12.01
	P	EP_A	17-May-18	10:37	74	78.7	12:40	2:03	123	100.05	12.31
	Q	EP_A	18-May-18	11:38	66.5	81.7	13:42	2:04	124	100.05	12.41
	R	EP_A	18-May-18	13:45	62.6	84.4	15:47	2:02	122	100.05	12.21
	S	EP_A	21-May-18	8:48	80.4	74.9	10:52	2:04	124	100.05	12.41
	T	EP_A	21-May-18	10:54	65	88.6	12:57	2:03	123	100.05	12.31
	U	EP_A	21-May-18	13:02	76.1	74.2	15:03	2:01	121	100.05	12.11

Table 3.1. Study type, run ID, sampler type (MF = membrane filtration, LI = liquid impinger, EP = electrostatic precipitator, and EP_A = electrostatic precipitator with agar plate), start time, humidity, temperature, end time, run duration, flow rate, and volume of air (m^3) pumped for the experiments.

Run ID	Sampler	Plate ID	Total CFUs	Bacterial CFUs	Fungal CFUs	Total CFUs m^{-3} air	Bacterial CFUs m^{-3} air	Fungal CFUs m^{-3} air
A	MF	A_MF	64	55	9	46.03	3.96	6.47
A	EP	A_EP	929	U	U	73.11	0.00	0.00
A	LI	A_LI	58	40	18	49.22	3.39	15.30
D	MF	D_MF	5	1	4	7.13	0.14	5.71
D	EP	D_EP	77	63	14	13.04	1.07	2.37
D	LI	D_LI	2	0	1	3.56	0.06	2.38
J	MF	J_MF	1	0	1	1.45	0.00	1.45
J	EP	J_EP	40	23	17	6.66	0.38	2.83
J	LI	J_LI	2	0	2	3.56	0.06	2.97
L	MF	L_MF	80	U	U	116.03	0.00	0.00
L	EP	L_EP	956	952	4	159.25	15.86	0.67
L	LI	L_LI	104	87	17	185.34	15.50	30.90
V	MF	V_MF	3	1	2	3.68	0.12	2.45
V	EP	V_EP	22	14	8	3.10	0.20	1.13
V	LI	V_LI	8	1	7	12.05	0.20	10.00

Table 3.2. Run ID, plate ID, total CFUs, bacterial CFUs, fungal CFUs, total CFUs m^{-3} air, bacterial CFUs m^{-3} air, fungal CFUs m^{-3} air, and aerosol collection method. (MF = membrane filtration, LI = liquid impinger, EP = electrostatic precipitator with no agar)

Run ID	Sampling Method	None	<0.1 (ug/mL)	≥=0.1 (ug/mL)
C	Membrane Filter		0.0355	
C	Liquid Impinger	Not Detectable		
C	Electrostatic Precipitator with no Agar	Not Detectable		
E	Membrane Filter		0.0214	
E	Liquid Impinger			0.11
E	Electrostatic Precipitator with no Agar	Not Detectable		
I	Membrane Filter		0.0205	
I	Liquid Impinger		0.02	
I	Electrostatic Precipitator with no Agar	Not Detectable		
K	Membrane Filter	Not Detectable		
K	Liquid Impinger		0.024	
K	Electrostatic Precipitator with no Agar		0.028	
M	Membrane Filter		0.014	
M	Liquid Impinger		0.023	
M	Electrostatic Precipitator with no Agar	Not Detectable		
P	Electrostatic Precipitator with no Agar		0.0877	
Q	Electrostatic Precipitator with Agar			0.2
R	Electrostatic Precipitator with Agar			0.121
S	Electrostatic Precipitator with Agar			0.11
T	Electrostatic Precipitator with Agar			0.15
U	Electrostatic Precipitator with Agar			0.244

Table 3.3. Run ID, sampler method, and DNA yield (ug/mL).

CONCLUSIONS & FUTURE WORK

The first two chapter of this thesis show the atmosphere can harbor a diverse array of microorganisms, which can have complex interactions with ambient marine microorganisms and can lead to changes in nutrient and carbon cycles. These two chapters add to the field of aeromicrobiology by demonstrating the ecological and biogeochemical importance of bioaerosols. These chapters are especially important because they focus on low-nutrient ecosystems, which are expanding. The last chapter contributes important data that will lead to standardization of sample collection techniques, without which comparison of results between results is hampered and the growth of the field may be stunted.

The second chapter of this thesis showed significant declines in *Synechococcus* abundance, eukaryotic alpha diversity, and primary productivity in the northern Red Sea (NRS) surface water in the presence of bioaerosols associated with dust. Future work should explore if these changes may be due to the introduction of organisms that compete for resources or predatory organisms or viruses through atmospheric deposition. Specifically, culture experiments should be carried out and focus on identifying the reasons why abundances of certain organisms, like *Synechococcus* in the NRS, are sensitive to atmospheric deposition. These experiments should incorporate virus enumeration and identification methods to investigate if viral lysis is the cause of declines in abundance. Quantification of trace metals to assess their role would also be useful.

The results from the second chapter also showed bacterial production and beta-glucosidase activity by heterotrophic bacteria increased with the addition of bioaerosols. These results may be explained by the addition of heterotrophic bacteria associated with dust particles that were introduced to the seawater during the mesocosm experiment, however this remains unresolved because we saw no significant differences between the organisms we found between the UV-killed and live dust treatments. There were 5 organisms identified at the family level that were present in the live dust treatments and absent in UV-killed dust treatments (Ruminococcaceae, Erysipelotrichaceae, Sphingomonadaceae, Xanthomonadacea, A4b), but their abundances were low (less than 25 sequences for each organism), yet these should be further studied. Alternatively, heterotrophic bacteria in the live dust treatments may have had increased access to nutrients due to the decline of primary producer populations and decreased primary productivity. Overall, the relationship between the variables we saw changes in (PP, BP, abundances, diversity) were poorly understood, and future work should focus on understanding how these variables are related and impact one another.