JGR Biogeosciences

RESEARCH ARTICLE
10.1029/2022JG007022

Functional Genes Profile of Atmospheric Dust in the East Mediterranean Suggests Widespread Anthropogenic Influence on Aerobiome Composition

Daniella Gat[1,2], Ralf Zimmermann[2,3], and Yinon Rudich[1]

[1]Department of Earth and Planetary Sciences, Weizmann Institute of Science, Rehovot, Israel, [2]Joint Mass Spectrometry Centre (JMSC) of Comprehensive Molecular Analytics (CMA), Helmholtz Zentrum München—German Research Center for Environmental Health GmbH, Munich, Germany, [3]Joint Mass Spectrometry Centre, Chair of Analytical Chemistry, Institute of Chemistry, University of Rostock, Rostock, Germany

Key Points:

- Atmospheric particles collected in Israel and in Saudi Arabia show resemblance in their bacterial composition and functional genes profile
- Airborne microorganisms show greater abundance of biodegradation genes than soils, sea and leaves
- Functional genes profile suggests anthropogenic impact on the atmospheric microbiome by way of pollution-associated bacteria

Supporting Information:

Supporting Information may be found in the online version of this article.

Correspondence to:

Y. Rudich,
yinnon.rudich@weizmann.ac.il

Citation:

Gat, D., Zimmermann, R., & Rudich, Y. (2022). Functional genes profile of atmospheric dust in the East Mediterranean suggests widespread anthropogenic influence on aerobiome composition. *Journal of Geophysical Research: Biogeosciences*, *127*, e2022JG007022. https://doi.org/10.1029/2022JG007022

Received 1 JUN 2022
Accepted 4 OCT 2022

Author Contributions:

Conceptualization: Daniella Gat, Yinon Rudich
Data curation: Daniella Gat
Formal analysis: Daniella Gat
Funding acquisition: Ralf Zimmermann, Yinon Rudich
Methodology: Daniella Gat
Resources: Ralf Zimmermann, Yinon Rudich
Supervision: Ralf Zimmermann, Yinon Rudich

Abstract The microbiome of atmospheric dust events has raised increasing interest in the last decade, resulting in numerous studies that characterized the different parameters affecting the composition of the atmospheric microbiome, that is, the aerobiome. However, less is known about the functional profile of the aerobiome and how it compares with other environments. Here, we describe the results of shotgun metagenome analysis conducted on a representative set of particulate matter (PM) samples taken in Israel under dusty and nondusty conditions. We compared the functional profiles of these samples to local metagenomes collected from soils, sea, and leaf surfaces and to PM collected in Saudi Arabia, in order to link between the sampled aerosols and potential sources that contribute to the aerobiome. We found that PM samples collected in Israel most resembled Saudi Arabian dust and Israeli soils in both community composition and functional genes profile. In addition, we found significant differences in the abundances of genes associated with anthropogenic activity. Specifically, the examined dust exhibited a significantly higher abundance of genes associated with the biodegradation of organic contaminants, mostly benzoate and aminobenzoate, compared with all other examined environments. These preliminary results suggest that an anthropogenic impact on the aerobiome composition and functional profile is widespread, and pave the path to understanding the role of dust storms in disseminating microorganisms in various environments, spreading various traits, and affecting humans, livestock, plants, and ecosystem health.

Plain Language Summary Microscopic particles such as dust and microorganisms are carried by winds and can travel great distances, crossing oceans and continents. The sources of airborne bacteria include soils, water bodies, plants, landfills, and livestock farms, among others. Different bacterial functions are associated with these different environments. Thus, examining the functional genes of airborne bacteria and comparing them to the surrounding environments can provide knowledge on the possible sources of the airborne bacteria and their potential functioning upon deposition. We compared the functional genes profile of atmospheric particles collected in Israel to those found in the surrounding seas, soils, leaf surfaces, and to dust collected in Saudi Arabia. We found that dust collected in Israel most resembled dust collected in Saudi Arabia, ~1,000 km away. We also identified bacteria and functional genes that were more abundant in Israeli and Saudi Arabian dust than in the examined environments. These included genes that allow the degradation of contaminants. These functional genes are usually found in association with human activity. We suggest that their presence in atmospheric particles collected in two distant locations indicates another aspect of human influence on the atmosphere that has to be further explored.

1. Introduction

For the past decade, data on global airborne microbial communities have been slowly accumulating (Bowers et al., 2011, 2013; Delort et al., 2010; Griffin, 2007; Katra et al., 2014; Lang-Yona et al., 2022; Mazar et al., 2016; Smith et al., 2013). A significant effort was invested in studying the taxonomic profiles of the atmospheric microbiome, that is, the aerobiome, by applying Next-Generation sequencing to atmospheric particulate matter (PM) samples. Low atmospheric DNA quantities have limited these efforts mainly to amplicon sequencing of a taxonomic proxy, such as the gene encoding the ribosome's small subunit (SSU), that is, 16S rRNA and 18S rRNA for prokaryotes and eukaryotes, respectively. This approach provided an important insight into the

Visualization: Daniella Gat
Writing – original draft: Daniella Gat
Writing – review & editing: Ralf Zimmermann, Yinon Rudich

microbial community structure and composition. It also offered some hints about the possible functionality of these microorganisms.

Fewer studies on the aerobiome have collected sufficient material to obtain the DNA concentrations required for metagenomic and metatranscriptomic sequencing. These studies explored different anthropogenic environments, such as farms and cities (Be et al., 2014; Cao et al., 2014; Tringe et al., 2008; Yooseph et al., 2013), natural environments in various geographic locations (Tignat-Perrier, Dommergue, Thollot, Magand, Vogel, et al., 2020), and different aerosol types, for example, cloud-condensation droplets (Amato et al., 2019) and atmospheric dust (Aalismail et al., 2019; Peng et al., 2021). The studies provided preliminary insights into the aerobiome's potential functions. Both studies by Aalismail et al. (2019) and Tignat-Perrier, Dommergue, Thollot, Magand, Vogel, et al. (2020) attempted to determine the potential survival of airborne microorganisms by looking at the abundance of specific functional genes that would render microorganisms adapted to atmospheric transport; these functions included resistance to UV radiation, oxidative stress, and sporulation. These genes were found in all air samples described in these studies yet were not always significantly more abundant in the aerobiome than in other microbiomes (Tignat-Perrier, Dommergue, Thollot, Magand, Vogel, et al., 2020). Once deposited, airborne bacteria were suggested to affect the functioning of local microbiomes (Rahav et al., 2020; Rahav, Ovadia, et al., 2016; Rahav, Paytan, et al., 2016) or affect local biota through the introduction of pathogens (Gonzalez-Martin et al., 2014; Maldonado-Ramirez et al., 2005; Prussin et al., 2015; Sharoni et al., 2015). However, studies describing these phenomena remain scarce.

Dust storms are a significant vector of microbial transport in the atmosphere, including the transport of potential plant and human pathogens (Griffin, 2007; Schmale & Ross, 2015). In the Eastern Mediterranean, the composition of microbial communities carried by dust differs from those found under no-dust conditions, with significant differences between the different dust sources, such as the Sahara desert and the Arabian Peninsula (Gat et al., 2017; Mazar et al., 2016). It was also shown that dust storms were characterized by an increased presence of soil bacteria and extremophiles (Gat et al., 2017; Mazar et al., 2016).

Israel is situated in the Eastern Mediterranean, at a crossroads of air masses from the Sahara, Arabian, and the Syrian deserts (Lelieveld et al., 2002). As such, it experiences dust plumes originating from all these sources. Its atmosphere exhibits a relatively high daily average loading of particulate matter under an aerodynamic diameter of 10 μm (PM_{10}), averaging approximately 30–50 μg m^{-3} and comprised of mineral dust (Krasnov et al., 2016), resulting in diverse dust-borne microbiome communities (Gat et al., 2017; Katra et al., 2014). This constant contribution of allochthonous microorganisms from the surrounding deserts into Israel's soils and the marine environment is interesting, especially if the imported microorganisms exhibit functions not commonly found in the local microbiomes. The rising frequency and intensity of dust events in the Eastern Mediterranean increase the imported microorganisms' chances of affecting the composition and function of local microbiomes.

To better define the differences in functional genes' profiles between dust and destination environments, that is, soils, the phyllosphere, and the sea, a comprehensive comparison of metagenomes was conducted. We applied shotgun metagenome sequencing to atmospheric PM_{10} sampled in Israel under different atmospheric PM_{10} concentrations and wind trajectories, representing various dust intensities and sources. These samples were compared to publicly available data sets of Israeli soils, the Mediterranean and the Red Seas, leaf surfaces of Athel trees (*Tamarix aphylla*) in Israel, and dust samples collected in Saudi Arabia. We compared the microbial community compositions and the functional genes profiles to find genes significantly more abundant in dust, thus providing the first clues for determining the possible effect of dust-admitted microorganisms on local microbiomes.

2. Materials and Methods

2.1. PM_{10} Sampling

Particulate matter was collected on the roof of a four-story building at the Weizmann Institute campus in Israel (31.9070°N, 34.8102°E) using a high-volume sampler (Ecotech, model: HiVol 3000, PM_{10} inlet, flow rate: 67.8 m^3 h^{-1}) onto prebaked quartz fiber filters (Whatman, 203 × 254 mm) for 6–48 hr at a time, depending on the load of particulate matter with an aerodynamic diameter under 10 μm (PM10). The filters were then wrapped in sterile aluminum foil and kept at −20 °C until DNA was extracted. The samples are described in Table 1. The source of each sample was determined using the hybrid single-particle Lagrangian integrated trajectory model (HYSPLIT) via a web-based interface (READY, http://ready.arl.noaa.gov/HYSPLIT_traj.php). The dust sources

Table 1
Dust Samples Collected in Israel

Sample	DNA concentration (pg μL^{-1})	Source	Sampling date	PM_{10} ($\mu g\ m^{-3}$)	Total air volume sampled (m^3)	Sampling duration (hr)
Dust_2	3,390	Local	18 July 2016	33	3,254	48
		North Africa	6 June 2016	35	3,254	48
		North Africa	15 August 2016	35	1,627	24
		Mix	31 August 2016	37	3,254	48
		Arabia	26 August 2016	40	2,983	44
Dust_3	1,390	North Africa	17 January 2017	75	3,254	48
		North Africa	9 May 2017	84	1,627	24
		North Africa	7 January 2017	96	3,254	48
		North Africa	20 November 2016	109	1,627	24
Dust_4	5,770	Mix (North Africa and Arabia)	25 November 2016	121	1,627	24
		North Africa	9 January 2017	144	3,254	48
		North Africa	12 March 2017	150	1,898	28
		Mix (North Africa and Arabia)	24 November 2016	186	3,254	48
Dust_5	3,270	North Africa	28 February 2016	209	2,034	30
		Mix (Sinai Peninsula and Jordan)	23 November 2016	232	1,424	21
		North Africa	12 April 2017	118	3,254	48
Dust_6	7,130	Syria	8 September 2015	844	949	14
Dust_7	10,200	Syria	9 September 2015	1859	983	14.5
Dust_8	3,490	Syria	10 September 2015	928	1,305	19.25
Dust_9	10,500	Arabia	3 November 2015	422	1,627	24
Dust_10	10,200	Arabia	4 November 2015	810	1,627	24

Note. Samples *Dust_2* to *Dust_5* were obtained by pooling DNA from several sampling events (after Peng et al. (2021)).

were also verified using the Giovanni online data system, developed and maintained by the NASA GES DISC (Acker & Leptoukh, 2007). Back trajectories for all sampling events detailed in Table 1 are presented in Table S1 in Supporting Information S1.

2.2. DNA Extraction

DNA was extracted using a DNA PowerSoil Kit (Qiagen, Germany), with modifications to the manufacturer's protocol as follows: four strips, 30 cm^2 each, were cut from each filter using a surgical scalpel, and the upper layer of each strip was scratched off and inserted into a single PowerSoil bead tube, to which 250 µL of PBS and 150 µL of a phenol:chloroform:isoamyl solution (ratio 25:24:1) were added. Next, the tubes were shaken in a Mini-Beadbeater-16 (Biospec, USA) for 2 min, removed and placed on ice for 15 s, and then shaken for an additional 2 min. Extraction continued according to the protocol up to the membrane-binding step. In this step, the contents of the four tubes were loaded onto two spin filter tubes. Next, the samples were washed twice with a C5 solution to rid the DNA of all traces of phenol and chloroform. Elution was conducted by loading 50 µL of solution C6 on one tube, leaving it for 5 min at room temperature, centrifuging for 1 min at 16,000× g, loading the eluent onto the second spin filter and repeating the described elution step. A blank filter used as a negative control was processed along with the samples.

Following extraction, samples with similar characteristics (PM_{10} concentration and source according to back-trajectories and satellite images) were pooled together (see Table 1), and DNA was precipitated using 100% ethanol and a 5 M NaCl solution. The precipitated DNA was resuspended in 25 µL of TE buffer. We thus created a set of nine samples, five representing two major dust events originating in Syria and in the Arabian Desert, three composite samples representing minor to moderate dust events from the Sahara, Sinai, and Jordanian deserts, and

Table 2
Metagenomes Used for Comparison With the Dust Collected in This Study

Metagenome data set	Description	Environment type	Reference and accession no.
Dust IL	Nine dust samples, Weizmann Institute, Israel	Dust	This study, mgp87645
Dust Red Sea	Eight dust samples, Red Sea, off the shore of Saudi Arabia	Dust	ENA, PRJEB31563 (Aalismail et al., 2019)
IL Soils	Twelve soil samples, different locations in Israel	Soil	mgp14608 (Tripathi et al., 2017)
Negev Soils	Five soil crust samples from the Negev desert, Israel	Soil	ENA, PRJEB36534 (Meier et al., 2021)
Red Sea	Four Red Sea water samples, different locations	Sea	mgp20413 (Sunagawa et al., 2015)
Med Sea	Six Mediterranean Sea water samples, different locations	Sea	mgp20252 (Kopf et al., 2015)
Leaves	Five samples of leaf-surface microbiome of *Tamarix aphylla*, from different locations in Israel	Leaf surface	mpg5802 (Finkel et al., 2016)

a single composite sample representing days with low PM_{10} concentrations. The pooling of samples was necessary due to low DNA yields.

2.3. Shotgun Metagenome Sequencing

Sequencing was conducted at the Genome Research Core of the University of Illinois, Chicago, as follows: DNA quantity and quality were assessed using a High Sensitivity D1000 ScreenTape (Agilent Technologies, Santa Clara, CA, USA); results are presented in Table 1. Blank control showed no DNA presence and was therefore omitted from further processing. After acoustic shearing, library preparation was performed using Accel-NGS 1S Kit (Swift Biosciences, Anne Arbor, MI, USA). The libraries were pooled, followed by Pippin-prep (Sage Science, Beverly, MA, USA) size selection (375–425 bp insert size). MiniSeq Q/C was performed to ensure an even distribution of sequencing output. The samples were repooled, followed by repeating the Pippin-prep size selection. The samples were subsequently sequenced by a HiSeq2500 (Illumina, San Diego, CA, USA) with 2×250 cycles using a single lane.

2.4. Metagenome Data Set Composition and Analysis

Following sequencing, adaptors and low-quality bases were removed from the raw reads using cutadapt v1.1 (Martin, 2011), reads shorter than 25 bases were removed. The trimmed reads were assembled independently using IDBA_UD (Peng et al., 2012), parameters set to default, contigs under 1,000 bases were discarded. We compared our PM_{10} samples to publicly available metagenomes representing Israeli soils, the Mediterranean and the Red Seas, and leaf surfaces of *Tamarix aphylla* collected at various locations in Israel, as well as to PM_{10} samples collected in Saudi Arabia, at the coast of the Red Sea. All data sets and environment types are described in Table 2. Raw and assembled reads of all described data sets were uploaded to the MG-RAST metagenomics analysis server (Keegan et al., 2016) and analyzed using the default parameters for shotgun metagenomic sequencing, for both taxonomy and functional genes annotation. Taxonomic data were acquired by comparison to the RefSeq database (NCBI (Brister et al., 2015; O'Leary et al., 2016; Tatusova et al., 2016)), and functional genes were annotated against the KO database (KEGG, Kanehisa & Goto, 2000; Minoru Kanehisa, 2019; Minoru Kanehisa et al., 2019), both available on the MG-RAST server. Taxonomic data were filtered to exclude multicellular organisms and viruses. Functional annotation data were filtered to exclude organismal systems and human disease categories. To account for possible contamination in samples with low DNA concentrations, we excluded all genera listed in Glassing et al. (2016) from the taxonomic analysis as suspected contaminants originating in DNA extraction kits and protocols. However, for functional genes, no such list was available; we thus assumed that PM_{10} samples collected under low atmospheric concentrations are more likely to be affected by such contamination. Accordingly, we correlated functional genes in Israeli Dust samples to PM_{10} concentrations. All genes that negatively correlated to PM_{10} concentrations were excluded from further analysis over the entire data set. Comparing PCA analysis of filtered taxa and gene lists with unfiltered lists revealed no differences between results; thus, all results presented were based on the filtered data sets.

Statistical analysis was conducted using R v.4.0.3 (R Core Team, 2020) and the R packages Vegan (Oksanen et al., 2007), tidyverse (Robinson et al., 2020), ggpubr (Kassambara, 2020), and ggplot2 (Wickham, 2016). To reduce matrix sparsity, we filtered out species and genes with a maximal count of 10 or lower per sample and a total of 30 or lower over the entire data set. To account for the compositionality of the data (Gloor et al., 2017; Gloor, Wu, et al., 2016; Quinn et al., 2018), taxa counts at the species level and gene counts were additive log-ratio transformed (alr, Greenacre et al., 2021) using the Compositions package (van den Boogaart, 2020) after handling zero counts by Geometric Bayesian multiplicative transformation, using the zCompositions package (Palarea-Albaladejo & Martín-Fernández, 2015). As a reference for alr-transformation, we used the counts of the *asd* (aspartate-semialdehyde dehydrogenase, EC: 1.2.1.11, K00133) gene, known as a universal single-copy gene with a mean length of ~1kb (Manor & Borenstein, 2015). Thus, alr-transformation also served as a normalization, allowing comparison between the different data sets (Quinn et al., 2019).

To identify differentially abundant genes and taxa, we applied the ALDEx2 package, using an interquartile log-ratio correction to account for a slight asymmetry between the data sets (Fernandes et al., 2013, 2014; Gloor, Macklaim, & Fernandes, 2016). Significance was defined as an absolute effect size greater than 1 and a Benjamini-Hochberg adjusted p value under 0.05 in both Welch's t test and Wilcoxon nonparametric test.

PCA, AMOVA, and PERMANOVA analyses were based on Euclidean distances. Analysis of molecular variance (AMOVA) (Excoffier et al., 1992) was conducted using the *amova* command in *mothur* v.1.45.3 (Schloss et al., 2009); NMDS ordinations were created in *mothur*, and were based on Bray-Curtis distances, calculated by repeated subsampling using Vegan package function *avg.dist()*; fitting of functional categories, taxa and PCA or NMDS axes were conducted using the *corr.axes* command in *mothur* with the method set to Spearman, all features presented in PCA and NMDS represent vectors with a relative length of over 80% and a p value under 0.05 for either one of the relevant ordination axes.

To assess the source apportionment of each environmental microbiome to the aerobiome we applied SourceTracker (Knights et al., 2011), using a biome file created by *mothur* based on unnormalized taxa count-tables, all parameters were set to default.

3. Results

3.1. Israeli Dust Community Composition and Functional Genes Profile

A biplot of principal component analysis (PCA) and significantly correlated functional genes and taxa in the *Dust_IL* samples is presented in Figure 1. Both PCAs showed a similar tendency to form three clusters: samples with low to medium PM_{10} concentrations, that is, *Dust_2* to *Dust_5*; Syrian dust storm *Dust_6* to *Dust_8*; and Arabian dust storm *Dust_9* and *Dust_10*. The similarity in clustering between community composition and functional gene profiles corroborates our previously raised hypothesis that differences in community composition between different dust sources suggest different functional gene profiles (Gat et al., 2017). Correlating KEGG annotations of metabolic activities to the PCA axes showed that more genes associated with metabolic activities correlated with the two sampled dust storms than with low PM_{10} samples, 185 versus Fifty-four genes, respectively. This is expected since we manually removed many functional genes that correlated with decreasing PM_{10} concentrations. The distribution of the different metabolic categories was generally similar for both dust storms and low PM_{10}, with the most of the genes related to amino acid metabolism (28% and 26%, respectively), followed by carbohydrate metabolism (21% and 22%, respectively). Yet, the metabolism of cofactors and vitamins was more dominant in genes associated with the Syrian dust storm than with low PM_{10} (18 versus 7%, respectively).

According to our results, mixed-source samples *Dust_2* to *Dust_5*, which represent lower PM_{10} concentrations and are primarily from western and southern trajectories, were associated with a higher abundance of Proteobacteria (gamma) and Firmicutes, along with the fungal phyla, Ascomycota and Basidiomycota. Dust samples collected in storms from the Syrian and Arabian deserts were primarily associated with Actinobacteria, Proteobacteria (alpha and delta), and the Archean phylum Euryarchaeota. Applying NMDS ordination on Bray-Curtis distances for the same data set produced similar results (Figure S2 in Supporting Information S1). These results are in agreement with Gat et al. (2017, 2021).

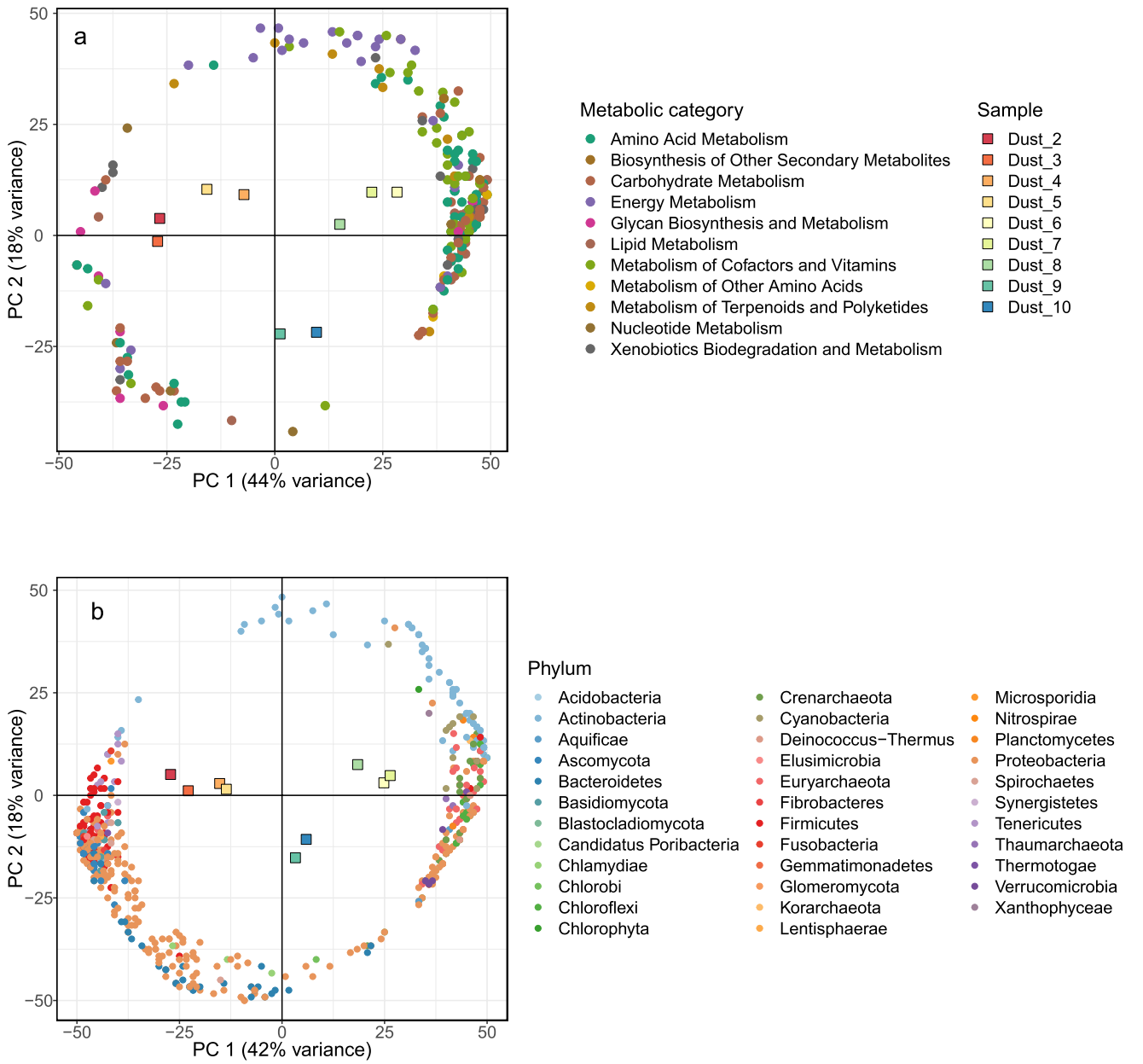

Figure 1. PCA biplot of PM_{10} samples collected in this study, based on their functional genes profile (a) and on their taxonomic composition (b).

3.2. Comparing PM_{10} Samples to Their Surrounding Environments

PM_{10} samples collected in Israel were compared to publicly available metagenomes collected from the Mediterranean and Red Seas, soils across Israel, leaf surfaces across Israel, and PM_{10} sampled in Saudi Arabia on the Red Sea shore (Table 1). Figure 2 shows principal component analyses of all data sets based on functional genes profile and community composition. In general, samples representing similar environments, for example, seas or soils, were clustered more closely, with the differences between data sets more pronounced in the functional profile than in the community composition. According to AMOVA tests, the community compositions and functional profiles of all examined environments significantly differed (Bonferroni pair-wise error rate under 0.001; Table S2 in Supporting Information S1). When reviewing the pair-wise differences between *Dust_IL* and the different data sets, all data sets' communities, except for *Dust_Red_Sea*, significantly differed from *Dust_IL* (Bonferroni pair-wise error rate over 0.001; Table S3 in Supporting Information S1), and all functional genes

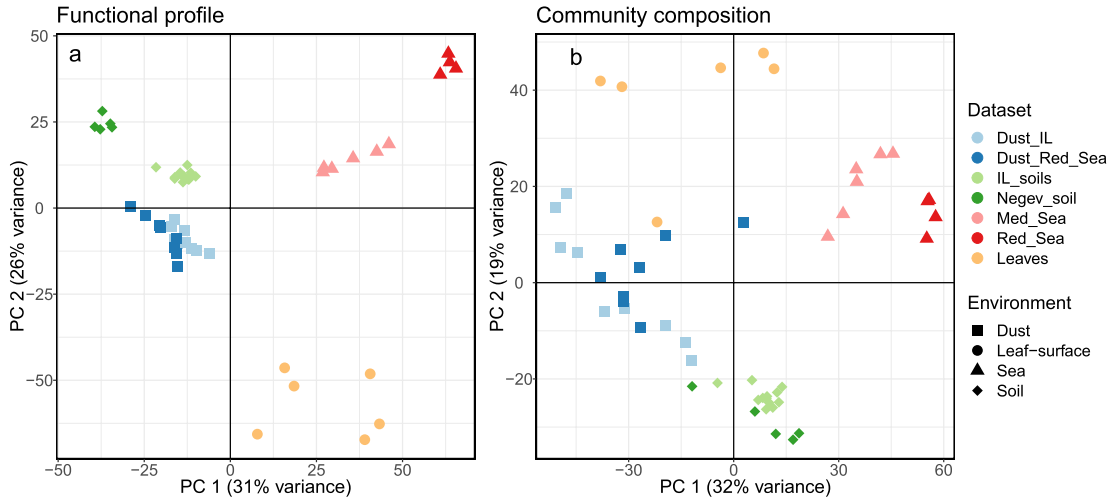

Figure 2. PCA of samples representing dust, leaf surfaces, sea, and soil, based on functional genes profile (a), and microbial community composition (b).

profiles differed from *Dust_IL* without exception (Bonferroni pair-wise error rate under 0.001; Table S3 in Supporting Information S1).

A PERMANOVA analysis confirmed these results, with significant differences between samples from the different environments and different studies ($p = 0.001$); in the functional profile, 49.5% of the variance was attributed to the environment and 27.3% to the different data sets, compared with 31.8% and 15.0%, respectively, in the community composition. Since the different studies sampled different locations and varied in their sampling procedures, DNA extraction, and sequencing methods, differences are expected.

We also looked for the potential contribution of each environment's community composition to the PM_{10} sampled in Israel by applying the SourceTracker algorithm to the taxonomic data set. The results are displayed in Figure 3. According to the results, Israeli soils, comprised of *IL_soils* and *Negev_soil*, and *Dust_Red_Sea* showed the largest potential contribution to *Dust_IL* samples, with a mean contribution of 34 ± 17% and 33 ± 7%, for soils and *Red_Sea_Dust*, respectively, followed by unknown sources (average of 20 ± 14%), leaf surface (average 11 ± 8%), and the sea (average 0.9 ± 0.3%). A PM_{10} increase in the Israeli data set was accompanied by an increase in the relative contribution of soils' microbiome to the community composition, for example, ~54% in all samples of the Syrian dust storm (*Dust_6* to *Dust_8*). The Arabian dust storm samples, *Dust_9* and *Dust_10*, were most influenced by *Dust_Red_Sea* (~43%), dust samples collected in Saudi Arabia. The relative contribution of

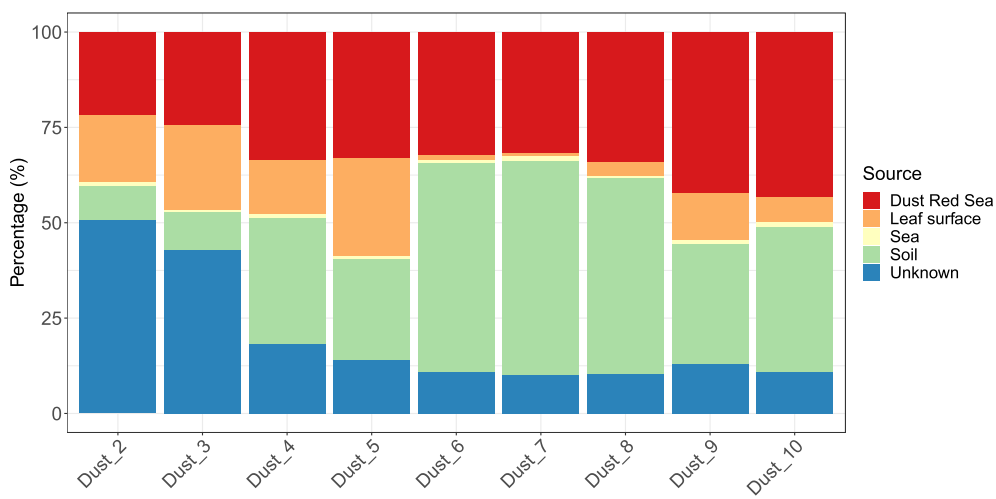

Figure 3. Putative contribution of the different environments to the *Dust_IL* microbiome, based on SourceTracker analysis. Samples *Dust_6* to *Dust_10* represent consecutive days on two major dust events.

the Mediterranean and Red Sea microbiomes to *Dust_IL* was generally very low, with a max of 1.3% contribution to *Dust_10*. Leaf surfaces were previously suggested as possible contributors to the aerobiome (Šantl-Temkiv et al., 2022; Tignat-Perrier et al., 2019). We found that the leaf surfaces most resembled the samples representing southern and western trajectories (*Dust_2* to *Dust_5*) with a maximal resemblance of 26%. This can also be attributed to the sampling period described in Finkel et al. (2016), which overlapped with some of the sampling events included in *Dust_IL* and may have included dust deposited on the sampled leaves.

3.3. Differential Abundance of Functional Genes in Dust Samples

Dust_IL samples were compared with the different environments, that is, sea, soil, and leaf surface, to detect the potential contribution of dust storms to local microbial functional genes profile. We also compared *Dust_IL* samples to *Dust_Red_Sea* samples collected by Aalismail et al. (2019) to better define how the two similar data sets differ. The differentially abundant genes were assigned to environmentally relevant functional categories (Table S4) (Escalas et al., 2019; Fierer et al., 2014). Figure 4 shows all genes that were significantly more abundant in *Dust_IL* than in the other examined data sets; full results are found in Table S5.

In total, 424 genes differed significantly between *Dust_IL* and *Dust_Red_Sea*, and 774 genes differed significantly between *Dust_IL* and soils. A greater number of genes differed significantly between *Dust_IL* and leaf surfaces and sea samples, 1,005 and 1,320, respectively (Table S5). Assigning functional traits to the different genes revealed that many of the significantly more abundant genes in *Dust_IL* were associated with biodegradation of organic compounds, such as benzoate, aminobenzoate, xylene, etc. Looking at the pair-wise comparisons between *Dust_IL* and the different environments, we found 55 and 56 genes of bioremediation to be more abundant in *Dust_IL* than in leaf-surface and sea samples, respectively. When compared with soils, 23 bioremediation-related genes were significantly more abundant in *Dust_IL*, compared with 16 genes that were more abundant in the soil microbiome. Only five genes associated with bioremediation of organic compounds were significantly more abundant in *Dust_IL* than in *Dust_Red_Sea*.

Genes associated with antimicrobial resistance (AMR) did not show a clear pattern of differential abundance between environments; different types of antibiotic resistance genes were either more or less abundant in dust, depending on the compared environment (Figure 4). Thus, *ampC*, associated with resistance to beta-lactam antibiotics, was more abundant in dust samples than in sea and leaf surface samples, but was not significantly more abundant compared with soils. In contrast, *pbpA*, also associated with beta-lactam resistance, was more abundant in dust samples than in soils, but not compared to leaf surfaces and seas. A total of seven AMR associated genes were significantly more abundant in *Dust_IL* than in any other environment, while a total of 12 AMR associated genes were significantly less abundant in *Dust_IL* than in the examined environments. Only two AMR associated genes were found to be differentially abundant between *Dust_IL* and *Dust_Red_Sea*.

Some carbon fixation genes, associated with different mechanisms, such as oxygenic photosynthesis or anoxygenic photosynthesis in purple bacteria, were also more abundant in *Dust_IL* than in the other environments. When comparing the number of genes significantly more abundant in *Dust_IL* than in other environments, we found that 27, 17, 10, and 9 carbon fixation genes were more abundant in *Dust_IL* than leaf surfaces, soils, the sea, and *Dust_Red_Sea* samples, respectively.

We found several stress resistance genes to be more common in dust samples in general and in *Dust_IL* in particular. Specifically, genes associated with oxidative stress were significantly more abundant in *Dust_IL* than in sea and soil samples; genes associated with osmotic stress were more abundant in *Dust_IL* than in all other data sets, except for *Dust_Red_Sea*. Genes associated with resilience to UV radiation were less abundant in *Dust_IL* than in all data sets but leaf surfaces. Finally, sporulation and extreme temperature resilience genes were more abundant in *Dust_IL* than in soils and the sea.

The *Dust_IL* samples exhibited significantly higher abundances of virulence-associated genes than sea and leaf-surface samples but lower than in soil samples. Among these genes were *devS* and *devR*, a two-component system related to the virulence of *Mycobacterium tuberculosis* (Dasgupta et al., 2000; Saini et al., 2004), and *rpfC*, *rpfG*, and *rpfF*, which are found in the phytopathogens of the genus *Xanthomonas* (An et al., 2013; Barber et al., 1997; Dow et al., 2003). Accordingly, we found that *Mycobacterium tuberculosis* were significantly more abundant in *Dust_IL* samples than in sea samples and on leaf surfaces (Table S6). No significant differences in virulence-associated gene abundance were found between *Dust_IL* and *Dust_Red_Sea* samples.

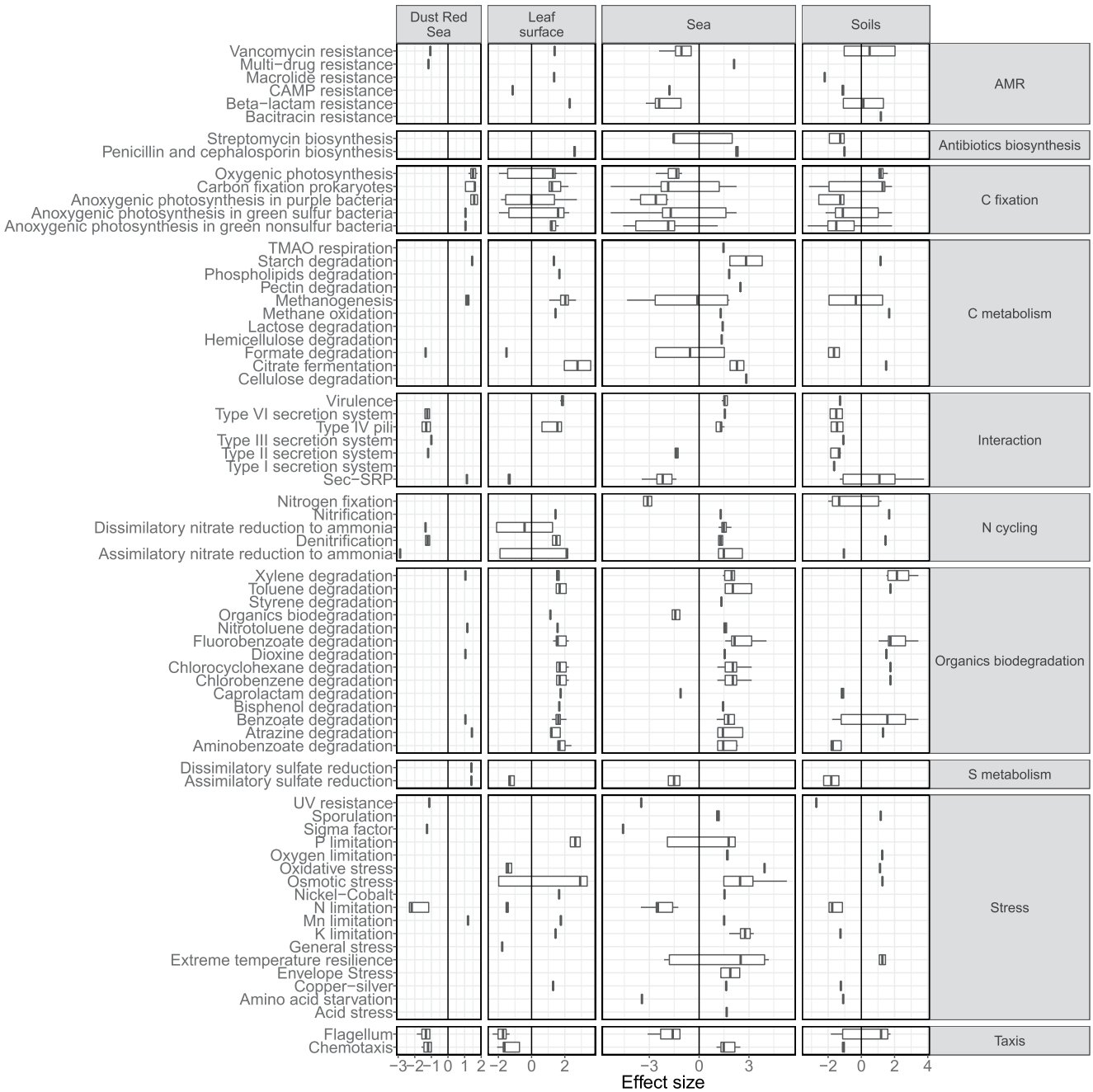

Figure 4. Genes exhibiting significantly different abundances in *Dust_IL* compared to other examined environments, presented by functional categories. Categories with a positive effect size are more abundant in *Dust_IL* than in other environments.

4. Discussion

Molecular-based studies of atmospheric samples are characterized by low DNA concentrations and are therefore prone to biases due to sample contamination (Salter et al., 2014). In this study, we used a blank filter extraction to identify contaminant species. However, the DNA concentration in the blank filter was too low to allow sequencing. Thus, we used published data on common contaminant taxa and excluded those from the taxonomic analysis. In addition, we assumed that low PM_{10} samples were richer in contaminant DNA and therefore correlated gene abundance to PM_{10} concentration and excluded all genes that negatively correlated significantly with the PM_{10} concentrations from the analysis. PCA analyses performed before and after removing potential contaminants did

not vary. While we presume these measures did not remove all contaminant DNA, we assume that they mitigated the expected bias.

The low biomass yield in some of the samples, which compelled us to group different samples prior to sequencing, limited our ability to compare the different air-mass sources and added a confounding effect to the comparison between low and high PM_{10} samples within the *Dust_IL* data set. In addition, variance in sampling duration might also affect the results to an unknown extent. Nonetheless, the measures we took to exclude any potential contaminant taxa and genes should mitigate the effect of these confounding variables. We thus examined the analysis of *Dust_IL* data set as a "sanity check," providing an assurance that these samples indeed resemble similar studies conducted in the same location, under similar conditions, and can therefore be reliably used to compare with other environmental samples.

Previous studies on atmospheric metagenome tried to determine whether a preselected set of genes were significantly enriched in atmospheric samples compared with other environments. The genes of choice were mainly associated with stress resistance (Aalismail et al., 2019; Tignat-Perrier, Dommergue, Thollot, Magand, Vogel, et al., 2020) and metabolism of compounds present in the atmosphere (Amato et al., 2019). Thus, the relative abundance of each gene was tested individually and, in some cases, compared to other environments. However, we could not find in the literature a comprehensive screening of atmospheric metagenomes and comparison with other environments using tools specifically designed for analysis of compositional data with corrections for multiple testing. Using this approach, we can now shed light on a greater variety of genes and functions transported during dust events in the Eastern Mediterranean.

We compared shotgun sequences of dust samples with sequences from other research groups representing different environments. These results are likely affected by different handling and sequencing methods and other batch effects. Nonetheless, we found significant clustering according to the environment in both community compositions and functional gene profiles. The variance between the different environments was significant, enabling us to detect genes and taxa significantly more abundant in dust samples than in the other environments.

4.1. Source-Sink Relationships and Similarity Between Dust and Other Environments' Microbiome

We compared the *Dust_IL* microbiome to soils from various locations in Israel and to the Mediterranean and Red Seas to find the possible contributions of dust to the microbiomes of the soils and seas in Israel and vice versa. Comparing dust samples collected in Saudi Arabia was designed to understand how the microbiome composition and functional genes profile of PM_{10} collected in Israel resemble other PM_{10} samples in the region. We first examined how different the dust was from these environments. According to PCA, AMOVA, and SourceTracker results, the microbiome identified in *Dust_IL* most resembled *Dust_Red_Sea*, sampled on different events over 1,000 km away from our sampling location, in both community composition and functional genes profile. Thus, fewer genes showed significantly differential abundance between the two PM_{10} data sets. Previous studies suggested that local land cover, that is, soil, vegetation, etc., is a dominant factor in determining the aerobiome composition (Bowers et al., 2011, 2013; Cáliz et al., 2018; Docherty et al., 2018; Els et al., 2019; Tignat-Perrier, Dommergue, Thollot, Magand, Amato, et al., 2020). Yet, the ability of dust storms to transfer particulate matter, mostly soil particles, from one location to another, on regional and global scales, leads to a greater contribution of the air-mass source to the aerobiome composition. Our results show a close link between different dust events in the East Mediterranean, further supporting the hypothesis that the air-mass source significantly affects this region's airborne microbial community composition.

Another environment that exhibited similarity to *Dust_IL* was local soil, as was shown previously in numerous studies sampling aerosols from different environments (Cao et al., 2014; Gat et al., 2017; Mazar et al., 2016; Tignat-Perrier, Dommergue, Thollot, Magand, Vogel, et al., 2020). The soils selected for comparison in this study were desert crusts from the Israeli Negev desert (*Negev_soil*) and various soils from different sites in Israel (*IL_soils*). It was previously shown that during a dust event, the most significant change in community composition compared with nondusty conditions is observed in the coarse particulate fraction, that is, >3 μm, representing particles and bacterial aggregates (Gat et al., 2021). Thus, the increasing similarity between soils and dust events with increasing PM_{10} concentrations is expected and further demonstrates the role of dust events in injecting soil microorganisms into the atmosphere, thereby affecting the aerobiome diversity and richness. In addition, we suggest an interplay between sources and sinks for soils and dust. The observed contribution of Israeli soils to

Dust_IL can result from the suspension of the local soil microbiome during dust events or the constant contributions of the dust-borne microbiome to the local soils via deposition. It can also result from the similarity between the soils at the origin of the dust event, that is, Syrian or Arabian Peninsula deserts, and local soils.

Another environment examined in this study was the marine environment, represented by two seas: the Mediterranean Sea and the Red Sea. Both seas are in the path of some of the air masses sampled in this study. Nonetheless, we found a very small contribution of the marine microbiome to the sampled aerobiomes. This result is in accordance with the results presented by Lang-Yona et al. (2022). An implication of the small overlap between the marine microbiome and the aerobiome is the contribution of dust deposition to aquatic environments. Dust can be considered a source for various microorganisms in the marine microbiome (Rahav, Ovadia, et al., 2016). Many of these microorganisms may not prevail after deposition due to incompatibility, predation, or a general inability to compete with the indigenous microbiome. Nonetheless, previous studies showed that aerosol deposition negatively affects Prochlorococcus communities in the Mediterranean Sea (Paytan et al., 2009; Rahav et al., 2020). In a changing climate, it is expected that the intensity and frequency of dust storms will increase, expanding the geographical range of the airborne bacteria, resulting in the introduction of dust-borne bacteria to previously pristine habitats, terrestrial and marine alike. It may increase the survival chances of the allochthonous bacteria in sink environments, specifically aquatic environments.

Leaf-top microbiomes showed a significant contribution to the composition of *Dust_IL* samples, according to SourceTracker results, and to the relatively lower number of differentially abundant genes between the two data sets. As in the case of soil and dust similarity, we could not determine whether this similarity results from a contribution of leaf microbiome to the aerobiome or vice versa.

4.2. Dust-Associated Functions

The majority of functional genes displaying differential abundance between *Dust_IL* and all other data sets were related to the biodegradation of various organic contaminants. Genes associated with the degradation of chlorocyclohexane, chlorobenzene, fluorobenzoate, toluene, and aminobenzoate, were significantly more abundant in *Dust_IL* than in sea, soil, and leaf surfaces metagenomes but not compared to *Dust_Red_Sea*, suggesting that the source of these genes is regional rather than local. Genes associated with benzoate, xylene, and atrazine degradation were significantly more abundant in *Dust_IL* than in all other examined data sets. We did not find a similar comparison made by any other metagenomic studies of atmospheric samples (Aalismail et al., 2019; Amato et al., 2019; Be et al., 2014; Cao et al., 2014; Tignat-Perrier, Dommergue, Thollot, Magand, Vogel, et al., 2020; Tringe et al., 2008; Yooseph et al., 2013), with the exception of Yooseph et al. (2013) that found some abundance of genes associated with xenobiotic biodegradation in air samples from urban aerosols. Some differentially abundant genes were found on bacterial plasmids, rendering them eligible for horizontal gene transfer (Leahy & Colwell, 1990; Perkins et al., 1990). Many of these genes were previously detected in contaminated soils and aquatic environments (Thelusmond et al., 2019), landfills, and bioreactors (Gupta et al., 2022; Yuan et al., 2017). Their abundance in dust samples from Israel and Saudi Arabia suggests that they are either enriched in the soils that contributed to the sampled dust events or that the anthropogenic impact on the aerobiome via aerosolization from landfills, wastewater treatment plants, or contaminated soils and water bodies, is ubiquitous in the East Mediterranean. Another possible explanation is that these genes provide their carrying microorganisms with an adaptation to the atmospheric habitat and are therefore relatively enriched, yet there is still insufficient data to support this hypothesis.

Dust_IL exhibited a higher abundance of some genes associated with antibiotic and antimicrobial resistance, yet comparing to different environments revealed a different pattern. Previous studies showed that antibiotic resistance genes (ARGs) are found in bioaerosols from various sources in different locations (Gat et al., 2017; Mazar et al., 2016; Wang et al., 2019; Zhu et al., 2021). Specifically, resistance to vancomycin and beta-lactam often cooccurred, and ARGs in the atmosphere positively correlated with air pollution (Wang et al., 2019; Zhu et al., 2021). This observation agrees with an ARGs survey in various environmental samples that showed that beta-lactam and, to a lesser extent, vancomycin resistance were ubiquitous yet more abundant in anthropogenic-related environments, such as livestock farms and human feces, than in natural soils, rivers, and sediments, by up to three orders of magnitude (Li et al., 2015). Suggested sources for ARGs in the atmosphere are wastewater treatment plants, livestock, and landfills (Wang et al., 2019; Yu et al., 2021).

Previous studies investigated whether the aerobiome is enriched in genes associated with resistance to UV radiation, extreme temperatures, oxidative stress, and sporulation-associated genes (Aalismail et al., 2019; Tignat-Perrier, Dommergue, Thollot, Magand, Vogel, et al., 2020). These traits were explicitly investigated as they are associated with microbial survival in harsh atmospheric conditions. Our results showed that *Dust_IL* samples displayed a significantly higher abundance of genes associated with sporulation, resistance to extreme temperatures, and oxidative stress than the examined soils and sea but not compared to leaf-surface metagenomes. Moreover, the examined soils and sea samples exhibited a significantly higher abundance of genes associated with resistance to UV radiation than the *Dust_IL*.

Intense dust plumes can protect transported microorganisms from atmospheric conditions, such as high UV radiation, and allow them to aggregate (Archer & Pointing, 2020; Meola et al., 2015; Šantl-Temkiv et al., 2022). Thus, dust-associated bacteria are more likely to survive their atmospheric transport and remain viable upon deposition.

5. Conclusions

Aerobiome composition is an important and understudied aspect of the anthropogenic impact on the atmosphere. As there is still very little knowledge on the role of the aerobiome in the planet's ecology, it is difficult to estimate how changes in its composition would affect environmental functioning. Thus, it is essential to continue the efforts to describe, record, and analyze the composition, functional profile, viability, and activity of the aerobiome. In this study we illustrate the important contribution of dust storms to the aerobiome composition. We suggest that this contribution can mask local effects caused by land cover. We found evidence to widespread anthropogenic effects on the aerobiome composition and functional profile, mostly in the form of significantly abundant genes associated with contaminants' biodegradation. We also show a great similarity between dust storms sampled at distant locations over different times, also suggesting that the anthropogenic impact on the aerobiome is of a regional scale. The global dispersion of dust and the accumulating evidence on its contribution to the viability of bacteria during atmospheric transport and data collected on the aerobiome's composition and functional profile highlights the possible impact of airborne bacteria on the destination environments.

Data Availability Statement

The sequencing data obtained in this study from sampled PM_{10} are available at MG-RAST server, project mgp87645: https://www.mg-rast.org/linkin.cgi?project=mgp87645.

Acknowledgments
The authors acknowledge Dr. Stefan Green of the University of Illinois, Chicago, for Illumina sequencing and technical counseling. We acknowledge Dr. Orit Altaratz-Stollar and Dr. Naama Reicher for assistance in producing and analyzing air-mass back-trajectories; Dr. Eddy Cytrin and Dr. Naama Lang-Yona for useful discussions and advice; and Dr. Avihai Zolti for guidance and assistance in metagenomic analysis. This study was partially supported by the Israel Science Foundation (Grant 236/16), the de Botton Center for Marine Science and the Helmholtz Zentrum München and Helmholtz Association (Berlin, Germany) in the framework of the aeroHEALTH Helmholtz International Lab (InterLabs-0005), a German-Israeli project. In this context, Daniella Gat thanks Helmholtz Zentrum München for funding her current position.

References

Aalismail, N. A., Ngugi, D. K., Díaz-Rúa, R., Alam, I., Cusack, M., & Duarte, C. M. (2019). Functional metagenomic analysis of dust-associated microbiomes above the Red Sea. *Scientific Reports*, *9*(1), 13741. https://doi.org/10.1038/s41598-019-50194-0

Acker, J. G., & Leptoukh, G. (2007). Online analysis enhances use of NASA Earth Science Data. *Eos Transactions American Geophysical Union*, *88*(2), 14. https://doi.org/10.1029/2007EO020003

Amato, P., Besaury, L., Joly, M., Penaud, B., Deguillaume, L., & Delort, A. M. (2019). Metatranscriptomic exploration of microbial functioning in clouds. *Scientific Reports*, *9*(1), 4383. https://doi.org/10.1038/s41598-019-41032-4

An, S.-Q., Febrer, M., McCarthy, Y., Tang, D.-J., Clissold, L., Kaithakottil, G., et al. (2013). High-resolution transcriptional analysis of the regulatory influence of cell-to-cell signalling reveals novel genes that contribute to Xanthomonas phytopathogenesis. *Molecular Microbiology*, *88*(6), 1058–1069. https://doi.org/10.1111/mmi.12229

Archer, S. D. J., & Pointing, S. B. (2020). Anthropogenic impact on the atmospheric microbiome. *Nature Microbiology*, *5*(2), 229–231. https://doi.org/10.1038/s41564-019-0650-z

Barber, C. E., Tang, J. L., Feng, J. X., Pan, M. Q., Wilson, T. J. G., Slater, H., et al. (1997). A novel regulatory system required for pathogenicity of *Xanthomonas campestris* is mediated by a small diffusible signal molecule. *Molecular Microbiology*, *24*(3), 555–566. https://doi.org/10.1046/j.1365-2958.1997.3721736.x

Be, N. A., Thissen, J. B., Fofanov, V. Y., Allen, J. E., Rojas, M., Golovko, G., et al. (2014). Metagenomic analysis of the airborne environment in urban spaces. *Microbial Ecology*, *69*(2), 346–355. https://doi.org/10.1007/s00248-014-0517-z

Bowers, R. M., Clements, N., Emerson, J. B., Wiedinmyer, C., Hannigan, M. P., & Fierer, N. (2013). Seasonal variability in bacterial and fungal diversity of the near-surface atmosphere. *Environmental Science & Technology*, *47*(21), 12097–12106. https://doi.org/10.1021/es402970s

Bowers, R. M., McLetchie, S., Knight, R., & Fierer, N. (2011). Spatial variability in airborne bacterial communities across land-use types and their relationship to the bacterial communities of potential source environments. *The ISME Journal*, *5*(4), 601–612. https://doi.org/10.1038/ismej.2010.167

Brister, J. R., Ako-Adjei, D., Bao, Y., & Blinkova, O. (2015). NCBI viral genomes resource. *Nucleic Acids Research*, *43*, D571–D577. https://doi.org/10.1093/nar/gku1207

Cáliz, J., Triadó-Margarit, X., Camarero, L., & Casamayor, E. O. (2018). A long-term survey unveils strong seasonal patterns in the airborne microbiome coupled to general and regional atmospheric circulations. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(48), 12229–12234. https://doi.org/10.1073/PNAS.1812826115

Cao, C., Jiang, W., Wang, B., Fang, J., Lang, J., Tian, G., et al. (2014). Inhalable microorganisms in Beijing's PM2.5 and PM10 pollutants during a severe smog event. *Environmental Science and Technology*, *48*(3), 1499–1507. https://doi.org/10.1021/es4048472

Dasgupta, N., Kapur, V., Singh, K. K., Das, T. K., Sachdeva, S., Jyothisri, K., & Tyagi, J. S. (2000). Characterization of a two-component system, devR-devS, of Mycobacterium tuberculosis. *Tubercle and Lung Disease*, *80*(3), 141–159. https://doi.org/10.1054/tuld.2000.0240

Delort, A. M., Vaïtilingom, M., Amato, P., Sancelme, M., Parazols, M., Mailhot, G., et al. (2010). A short overview of the microbial population in clouds: Potential roles in atmospheric chemistry and nucleation processes. *Atmospheric Research*, *98*(2–4), 249–260. https://doi.org/10.1016/j.atmosres.2010.07.004

Docherty, K. M., Pearce, D. S., Lemmer, K. M., & Hale, R. L. (2018). Distributing regionally, distinguishing locally: Examining the underlying effects of local land use on airborne bacterial biodiversity. *Environmental Microbiology*, *20*(10), 3529–3542. https://doi.org/10.1111/1462-2920.14307

Dow, J. M., Crossman, L., Findlay, K., He, Y.-Q., Feng, J.-X., & Tang, J.-L. (2003). Biofilm dispersal in Xanthomonas campestris is controlled by cell-cell signaling and is required for full virulence to plants. *Proceedings of the National Academy of Sciences of the United States of America*, *100*(19), 10995–11000. https://doi.org/10.1073/PNAS.1833360100

Els, N., Larose, C., Baumann-Stanzer, K., Tignat-Perrier, R., Keuschnig, C., Vogel, T. M., & Sattler, B. (2019). Microbial composition in seasonal time series of free tropospheric air and precipitation reveals community separation. *Aerobiologia*, *35*(4), 671–701. https://doi.org/10.1007/s10453-019-09606-x

Escalas, A., Hale, L., Voordeckers, J. W., Yang, Y., Firestone, M. K., Alvarez-Cohen, L., & Zhou, J. (2019). Microbial functional diversity: From concepts to applications. *Ecology and Evolution*, *9*(20), 12000–12016. https://doi.org/10.1002/ece3.5670

Excoffier, L., Smouse, P. E., & Quattro, J. M. (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. *Genetics*, *131*(2), 479–491. https://doi.org/10.1093/genetics/131.2.479

Fernandes, A. D., Macklaim, J. M., Linn, T. G., Reid, G., & Gloor, G. B. (2013). ANOVA-like differential expression (ALDEx) analysis for mixed population RNA-seq. *PLoS One*, *8*(7), e67019. https://doi.org/10.1371/journal.pone.0067019

Fernandes, A. D., Reid, J. N. S., Macklaim, J. M., McMurrough, T. A., Edgell, D. R., & Gloor, G. B. (2014). Unifying the analysis of high-throughput sequencing datasets: Characterizing RNA-seq, 16S rRNA gene sequencing and selective growth experiments by compositional data analysis. *Microbiome*, *2*(1), 15. https://doi.org/10.1186/2049-2618-2-15

Fierer, N., Barberán, A., & Laughlin, D. C. (2014). Seeing the forest for the genes: Using metagenomics to infer the aggregated traits of microbial communities. *Frontiers in Microbiology*, *5*, 614. https://doi.org/10.3389/fmicb.2014.00614

Finkel, O. M., Delmont, T. O., Post, A. F., & Belkin, S. (2016). Metagenomic signatures of bacterial adaptation to life in the phyllosphere of a salt-secreting desert tree. *Applied and Environmental Microbiology*, *82*(9), 2854–2861. https://doi.org/10.1128/AEM.00483-16

Gat, D., Mazar, Y., Cytryn, E., & Rudich, Y. (2017). Origin-dependent variations in the atmospheric microbiome community in Eastern mediterranean dust storms. *Environmental Science and Technology*, *51*(12), 6709–6718. https://doi.org/10.1021/acs.est.7b00362

Gat, D., Reicher, N., Schechter, S., Alayof, M., Tarn, M. D., Wyld, B. V., et al. (2021). Size-resolved community structure of bacteria and Fungi transported by dust in the Middle East. *Frontiers in Microbiology*, *12*, 744117. https://doi.org/10.3389/fmicb.2021.744117

Glassing, A., Dowd, S. E., Galandiuk, S., Davis, B., & Chiodini, R. J. (2016). Inherent bacterial DNA contamination of extraction and sequencing reagents may affect interpretation of microbiota in low bacterial biomass samples. *Gut Pathogens*, *8*(1), 24. https://doi.org/10.1186/s13099-016-0103-7

Gloor, G. B., Macklaim, J. M., & Fernandes, A. D. (2016). Displaying variation in large datasets: Plotting a visual summary of effect sizes. *Journal of Computational & Graphical Statistics*, *25*(3), 971–979. https://doi.org/10.1080/10618600.2015.1131161

Gloor, G. B., Macklaim, J. M., Pawlowsky-Glahn, V., & Egozcue, J. J. (2017). Microbiome datasets are compositional: And this is not optional. *Frontiers in Microbiology*, *8*, 2224. https://doi.org/10.3389/fmicb.2017.02224

Gloor, G. B., Wu, J. R., Pawlowsky-Glahn, V., & Egozcue, J. J. (2016). It's all relative: Analyzing microbiome data as compositions. *Annals of Epidemiology*, *26*(5), 322–329. https://doi.org/10.1016/j.annepidem.2016.03.003

Gonzalez-Martin, C., Teigell-Perez, N., Valladares, B., & Griffin, D. W. (2014). The global dispersion of pathogenic microorganisms by dust storms and its relevance to agriculture. *Advances in Agronomy*, *127*, 1–41. https://doi.org/10.1016/B978-0-12-800131-8.00001-7

Greenacre, M., Martínez-Álvaro, M., & Blasco, A. (2021). Compositional data analysis of microbiome and any-omics datasets: A validation of the additive Logratio transformation. *Frontiers in Microbiology*, *12*, 727398. https://doi.org/10.3389/fmicb.2021.727398

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

Gupta, J., Rathour, R., Dupont, C., Mishra, A., & Shekhar Thakur, I. (2022). Biogeochemical profiling and taxonomic characterization of municipal landfill site by metagenomic sequencing. *Bioresource Technology*, *351*, 126936. https://doi.org/10.1016/j.biortech.2022.126936

Kanehisa, M. (2019). Toward understanding the origin and evolution of cellular organisms. *Protein Science*, *28*(11), 1947–1951. https://doi.org/10.1002/pro.3715

Kanehisa, M., & Goto, S. (2000). Kegg: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Research*, *28*(1), 27–30. https://doi.org/10.1093/nar/28.1.27

Kanehisa, M., Sato, Y., Furumichi, M., Morishima, K., & Tanabe, M. (2019). New approach for understanding genome variations in KEGG. *Nucleic Acids Research*, *47*(D1), D590–D595. https://doi.org/10.1093/nar/gky962

Kassambara, A. (2020). Ggpubr: "ggplot2" based publication ready plots. Retrieved from https://cran.r-project.org/package=ggpubr

Katra, I., Arotsker, L., Krasnov, H., Zaritsky, A., Kushmaro, A., & Ben-Dov, E. (2014). Richness and diversity in dust stormborne biomes at the southeast Mediterranean. *Scientific Reports*, *4*(4), 5265. https://doi.org/10.1038/srep05265

Keegan, K. P., Glass, E. M., & Meyer, F. (2016). RAST, a metagenomics service for analysis of microbial community structure and function. *Methods in Molecular Biology*, *1399*, 207–233. https://doi.org/10.1007/978-1-4939-3369-3_13

Knights, D., Kuczynski, J., Charlson, E. S., Zaneveld, J., Mozer, M. C., Collman, R. G., et al. (2011). Bayesian community-wide culture-independent microbial source tracking. *Nature Methods*, *8*(9), 761–763. https://doi.org/10.1038/nmeth.1650

Kopf, A., Bicak, M., Kottmann, R., Schnetzer, J., Kostadinov, I., Lehmann, K., et al. (2015). The ocean sampling day consortium. *GigaScience*, *4*(1), 27. https://doi.org/10.1186/s13742-015-0066-5

Krasnov, H., Katra, I., & Friger, M. (2016). Increase in dust storm related PM10 concentrations: A time series analysis of 2001–2015. *Environmental Pollution*, *213*, 36–42. https://doi.org/10.1016/j.envpol.2015.10.021

Lang-Yona, N., Flores, J. M., Haviv, R., Alberti, A., Poulain, J., Belser, C., et al. (2022). Terrestrial and marine influence on atmospheric bacterial diversity over the north Atlantic and Pacific Oceans. *Communications Earth & Environment*, *3*(1), 121. https://doi.org/10.1038/s43247-022-00441-6

Leahy, J. G., & Colwell, R. R. (1990). Microbial degradation of hydrocarbons in the environment. *Microbiological Reviews*, *54*(3), 305–315. https://doi.org/10.1128/mr.54.3.305-315.1990

Lelieveld, J., Berresheim, H., Borrmann, S., Crutzen, P. J., Dentener, F. J., Fischer, H., et al. (2002). Global air pollution crossroads over the Mediterranean. *Science*, *298*(5594), 794–799. https://doi.org/10.1126/science.1075457

Li, B., Yang, Y., Ma, L., Ju, F., Guo, F., Tiedje, J. M., & Zhang, T. (2015). Metagenomic and network analysis reveal wide distribution and co-occurrence of environmental antibiotic resistance genes. *The ISME Journal*, *9*(11), 2490–2502. https://doi.org/10.1038/ismej.2015.59

Maldonado-Ramirez, S. L., Schmale, D. G. I., Shields, E. J., & Bergstrom, G. C. (2005). The relative abundance of viable spores of *Gibberella zeae* in the planetary boundary layer suggests the role of long-distance transport in regional epidemics of *Fusarium* head blight. *Agricultural and Forest Meteorology*, *132*(1), 20–27. https://doi.org/10.1016/j.agrformet.2005.06.007

Manor, O., & Borenstein, E. (2015). MUSiCC: A marker genes based framework for metagenomic normalization and accurate profiling of gene abundances in the microbiome. *Genome Biology*, *16*(1), 53. https://doi.org/10.1186/s13059-015-0610-8

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet Journal*, *17*(1), 10–12. https://doi.org/10.14806/ej.17.1.200

Mazar, Y., Cytryn, E., Erel, Y., & Rudich, Y. (2016). Effect of dust storms on the atmospheric microbiome in the eastern Mediterranean. *Environmental Science & Technology*, *50*(8), 4194–4202. https://doi.org/10.1021/acs.est.5b06348

Meier, D. V., Imminger, S., Gillor, O., & Woebken, D. (2021). Distribution of mixotrophy and desiccation survival mechanisms across microbial genomes in an arid biological soil crust community. *mSystems*, *6*(1), e00786-20. https://doi.org/10.1128/mSystems.00786-20

Meola, M., Lazzaro, A., & Zeyer, J. (2015). Bacterial composition and survival on Sahara dust particles transported to the European Alps. *Frontiers in Microbiology*, *16*, 1454. https://doi.org/10.3389/fmicb.2015.01454

Oksanen, J., Blanchet, F., Guillaume Kindt, R., Legendre, P., O'hara, R. B., Simpson, G. L., et al. (2007). Vegan: Community ecology package (R Package Version 1.17-4).

O'Leary, N. A., Wright, M. W., Brister, J. R., Ciufo, S., Haddad, D., McVeigh, R., et al. (2016). Reference sequence (RefSeq) database at NCBI: Current status, taxonomic expansion, and functional annotation. *Nucleic Acids Research*, *44*(D1), D733–D745. https://doi.org/10.1093/nar/gkv1189

Palarea-Albaladejo, J., & Martín-Fernández, J. A. (2015). zCompositions—R package for multivariate imputation of left-censored data under a compositional approach. *Chemometrics and Intelligent Laboratory Systems*, *143*, 85–96. https://doi.org/10.1016/j.chemolab.2015.02.019

Paytan, A., Mackey, K. R. M., Chen, Y., Lima, I. D., Doney, S. C., Mahowald, N., et al. (2009). Toxicity of atmospheric aerosols on marine phytoplankton. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(12), 4601–4605. https://doi.org/10.1073/pnas.0811486106

Peng, X., Gat, D., Paytan, A., & Rudich, Y. (2021). The response of airborne mycobiome to dust storms in the eastern Mediterranean. *Journal of Fungi*, *7*(10), 802. https://doi.org/10.3390/jof7100802

Peng, Y., Leung, H. C. M., Yiu, S. M., & Chin, F. Y. L. (2012). IDBA-UD: A de novo assembler for single-cell and metagenomic sequencing data with highly uneven depth. *Bioinformatics*, *28*(11), 1420–1428. https://doi.org/10.1093/bioinformatics/bts174

Perkins, E. J., Gordon, M. P., Caceres, O., & Lurquin, P. F. (1990). Organization and sequence analysis of the 2,4-dichlorophenol hydroxylase and dichlorocatechol oxidative operons of plasmid pJP4. *Journal of Bacteriology*, *172*(5), 2351–2359. https://doi.org/10.1128/jb.172.5.2351-2359.1990

Prussin, A. J., Marr, L. C., Schmale, D. G. I., Stoll, R., & Ross, S. D. (2015). Experimental validation of a long-distance transport model for plant pathogens: Application to *Fusarium graminearum*. *Agricultural and Forest Meteorology*, *203*, 118–130. https://doi.org/10.1016/j.agrformet.2014.12.009

Quinn, T. P., Erb, I., Gloor, G., Notredame, C., Richardson, M. F., & Crowley, T. M. (2019). A field guide for the compositional analysis of any-omics data. *GigaScience*, *8*(9), giz107. https://doi.org/10.1093/gigascience/giz107

Quinn, T. P., Erb, I., Richardson, M. F., & Crowley, T. M. (2018). Understanding sequencing data as compositions: An outlook and review. *Bioinformatics*, *34*(16), 2870–2878. https://doi.org/10.1093/bioinformatics/bty175

Rahav, E., Ovadia, G., Paytan, A., & Herut, B. (2016). Contribution of airborne microbes to bacterial production and N2 fixation in seawater upon aerosol deposition. *Geophysical Research Letters*, *43*, 719–727. https://doi.org/10.1002/2015GL066898

Rahav, E., Paytan, A., Chien, C.-T., Ovadia, G., Katz, T., & Herut, B. (2016). The impact of atmospheric dry deposition associated microbes on the Southeastern Mediterranean Sea surface water following an Intense dust storm. *Frontiers in Marine Science*, *3*, 127. https://doi.org/10.3389/fmars.2016.00127

Rahav, E., Paytan, A., Mescioglu, E., Bar-Zeev, E., Martínez Ruiz, F., Xian, P., & Herut, B. (2020). Bio-aerosols negatively affect Prochlorococcus in oligotrophic aerosol-rich marine regions. *Atmosphere*, *11*(5), 540. https://doi.org/10.3390/atmos11050540

R Core Team. (2020). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. Retrieved from https://www.r-project.org/

Robinson, D., Hayes, A., Gomez, M., Demeshev, B., Menne, D., Nutter, B., et al. (2020). Convert statistical analysis objects into tidy tibbles. Retrieved from http://github.com/tidyverse/broom

Saini, D. K., Malhotra, V., Dey, D., Pant, N., Das, T. K., & Tyagi, J. S. (2004). DevR-DevS is a bona fide two-component system of *Mycobacterium tuberculosis* that is hypoxia-responsive in the absence of the DNA-binding domain of DevR. *Microbiology (Reading, England)*, *150*(Pt 4), 865–875. https://doi.org/10.1099/mic.0.26218-0

Salter, S. J., Cox, M. J., Turek, E. M., Calus, S. T., Cookson, W. O., Moffatt, M. F., et al. (2014). Reagent and laboratory contamination can critically impact sequence-based microbiome analyses. *BMC Biology*, *12*(1), 87. https://doi.org/10.1186/s12915-014-0087-z

Šantl, Temkiv, T., Amato, P., Casamayor, E. O., Lee, P. K. H., & Pointing, S. B. (2022). Microbial ecology of the atmosphere. *FEMS Microbiology Reviews*, *46*(4), fuac009. https://doi.org/10.1093/femsre/fuac009

Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., et al. (2009). Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology*, *75*(23), 7537–7541. https://doi.org/10.1128/AEM.01541-09

Schmale, D. G. I., & Ross, S. D. (2015). Highways in the sky: Scales of atmospheric transport of plant pathogens. *Annual Review of Phytopathology*, *53*(1), 591–611. https://doi.org/10.1146/annurev-phyto-080614-115942

Sharoni, S., Trainic, M., Schatz, D., Lehahn, Y., Flores, M. J., Bidle, K. D., et al. (2015). Infection of phytoplankton by aerosolized marine viruses. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(21), 6643–6647. https://doi.org/10.1073/pnas.1423667112

Smith, D. J., Timonen, H. J., Jaffe, D. A., Griffin, D. W., Birmele, M. N., Perry, K. D., et al. (2013). Intercontinental dispersal of bacteria and archaea by transpacific winds. *Applied and Environmental Microbiology*, *79*(4), 1134–1139. https://doi.org/10.1128/AEM.03029-12

Sunagawa, S., Coelho, L. P., Chaffron, S., Kultima, J. R., Labadie, K., Salazar, G., et al. (2015). Structure and function of the global ocean microbiome. *Science*, *348*(6237), 1261359. https://doi.org/10.1126/science.1261359

Tatusova, T., DiCuccio, M., Badretdin, A., Chetvernin, V., Nawrocki, E. P., Zaslavsky, L., et al. (2016). NCBI prokaryotic genome annotation pipeline. *Nucleic Acids Research*, *44*(14), 6614–6624. https://doi.org/10.1093/nar/gkw569

Thelusmond, J.-R., Strathmann, T. J., & Cupples, A. M. (2019). Carbamazepine, triclocarban and triclosan biodegradation and the phylotypes and functional genes associated with xenobiotic degradation in four agricultural soils. *Science of The Total Environment*, *657*, 1138–1149. https://doi.org/10.1016/j.scitotenv.2018.12.145

Tignat-Perrier, R., Dommergue, A., Thollot, A., Keuschnig, C., Magand, O., Vogel, T. M., & Larose, C. (2019). Global airborne microbial communities controlled by surrounding landscapes and wind conditions. *Scientific Reports*, *9*(1), 14441. https://doi.org/10.1038/s41598-019-51073-4

Tignat-Perrier, R., Dommergue, A., Thollot, A., Magand, O., Amato, P., Joly, M., et al. (2020). Seasonal shift in airborne microbial communities. *Science of the Total Environment*, *716*, 137129. https://doi.org/10.1016/j.scitotenv.2020.137129

Tignat-Perrier, R., Dommergue, A., Thollot, A., Magand, O., Vogel, T. M., & Larose, C. (2020). Microbial functional signature in the atmospheric boundary layer. *Biogeosciences*, *17*(23), 6081–6095. https://doi.org/10.5194/bg-17-6081-2020

Tringe, S. G., Zhang, T., Liu, X., Yu, Y., Lee, W. H., Yap, J., et al. (2008). The airborne metagenome in an Indoor urban environment. *PLoS One*, *3*(4), e1862. https://doi.org/10.1371/journal.pone.0001862

Tripathi, B. M., Moroenyane, I., Sherman, C., Lee, Y. K., Adams, J. M., & Steinberger, Y. (2017). Trends in taxonomic and functional composition of soil microbiome along a precipitation Gradient in Israel. *Microbial Ecology*, *74*(1), 168–176. https://doi.org/10.1007/s00248-017-0931-0

van den Boogaart, K. G. (2020). Compositions: Compositional data analysis. Retrieved from https://cran.r-project.org/package=compositions

Wang, Y., Wang, C., & Song, L. (2019). Distribution of antibiotic resistance genes and bacteria from six atmospheric environments: Exposure risk to human. *Science of The Total Environment*, *694*, 133750. https://doi.org/10.1016/j.scitotenv.2019.133750

Wickham, H. (2016). *ggplot2: Elegant graphics for data analysis*. Springer International Publishing. Retrieved from https://ggplot2.tidyverse.org

Yooseph, S., Andrews-Pfannkoch, C., Tenney, A., McQuaid, J., Williamson, S., Thiagarajan, M., et al. (2013). A metagenomic framework for the study of airborne microbial communities. *PLoS One*, *8*(12), e81862. https://doi.org/10.1371/journal.pone.0081862

Yu, Y., Liang, Z., Liao, W., Ye, Z., Li, G., & An, T. (2021). Contributions of meat waste decomposition to the abundance and diversity of pathogens and antibiotic-resistance genes in the atmosphere. *Science of The Total Environment*, *784*, 147128. https://doi.org/10.1016/j.scitotenv.2021.147128

Yuan, K., Chen, B., Qing, Q., Zou, S., Wang, X., & Luan, T. (2017). Polycyclic aromatic hydrocarbons (PAHs) enrich their degrading genera and genes in human-impacted aquatic environments. *Environmental Pollution*, *230*, 936–944. https://doi.org/10.1016/j.envpol.2017.07.059

Zhu, G., Wang, X., Yang, T., Su, J., Qin, Y., Wang, S., et al. (2021). Air pollution could drive global dissemination of antibiotic resistance genes. *The ISME Journal*, *15*(1), 270–281. https://doi.org/10.1038/s41396-020-00780-2