SCIENTIFIC REPORTS

OPEN

SUBJECT AREAS:
ENVIRONMENTAL SCIENCES
AIR MICROBIOLOGY

Received
11 February 2014

Accepted
15 May 2014

Published
12 June 2014

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Richness and Diversity in Dust Stormborne Biomes at the Southeast Mediterranean

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Dust storms include particulate matter that is transported over land and sea with biota that could impact downwind ecosystems. In addition to the physico-chemical compositions, organismal diversities of dust from two storm events in southern Israel, December 2012 (Ev12) and January 2013 (Ev13), were determined by pyro-sequencing using primers universal to 16S and 18S rRNA genes and compared. The bio-assemblages in the collected dust samples were affiliated with scores of different taxa. Distinct patterns of richness and diversity of the two events were influenced by the origins of the air masses: Ev13 was rich with reads affiliated to *Betaproteobacteria* and *Embryophyta*, consistent with a European origin. Ev12, originated in north-Africa, contained significantly more of the *Actinobacteria* and fungi, without conifers. The abundance of bacterial and eukaryotic reads demonstrates dissemination of biological material in dust that may impose health hazards of pathogens and allergens, and influence vegetation migration throughout the world.

Dust storms, considered major contributors to global aerosols[1], transport desert soils to the atmosphere and substantially impact the global environment. Estimates of global dust emissions from soils to the atmosphere vary between 1 and 3 pg per year[2]. Climatic variations affect the land surfaces of aeolian (wind-driven) systems[3], and droughts have significantly contributed to increased dust emissions[4,5]. The contribution of anthropogenic soils, e.g. agricultural fields and industrial yards, are of increasing concern[6]. Up to 50% of the total atmospheric dust originates from disturbed soils[7], which may contain different particle compositions from that of natural dust. The windblown dust can travel tens of thousands of kilometers before being deposited[1], depending on the particle characteristics (size, chemistry) and the air-mass properties (e.g., velocity, density, height)[8]. The size and chemical compositions vary in space and time and may determine the potential impact on air quality and human health[9,10].

Airborne dust particles contain different microorganisms that are exceedingly mobile in space and time[11,12]. For example, the number of culturable microorganisms in the US Virgin Islands increased during African dust-storm events by about 8 fold, from 0.013 L^{-1} of air under clear atmospheric conditions to 0.105[13,14]. Dust storms originating in the Saharan desert heavily affect the south-eastern Mediterranean basin, mostly during the winter and spring[15]; qualitative and quantitative analyses of dust-associated fungal communities revealed distinct pattern of distribution in the atmosphere of Haifa (Israel)[16,17].

Airborne dust contains a variety of chemicals and microbial agents such as bacteria, fungi, and viruses where some of them are pathogenic and pose a risk to the ecosystem and human health as the clouds traverse regions[11,12,18]. The composition of microorganisms is still not well-defined, and taxonomic studies of organisms' diversity in the outdoor air have just started to emerge. In recent years, conventional molecular approaches have widely been used to study the diversity and community composition of prokaryotes and eukaryotes in air and outdoor dusts[17,19–24], but the methods exploited are incomplete. Clone libraries, Restriction Fragment Length Polymorphism, Denaturative Gradient Gel Electrophoresis, Ribosomal Intergenic Spacer Analysis and PCR-single Strand Conformation Polymorphism are usually restricted to less than 500 sequences or patterns. These

approaches, albeit providing general information on the structure of explored communities, are therefore not sufficient for meaningful comparisons.

In this study, the high throughput sequencing method (pyrosequencing) was used to explore, the structure of the prokaryotic and eukaryotic communities in airborne samples of dust collected in an urban environment in the Negev (southern Israel) following two distinct desert storm events.

Results and Discussion

The arena. Samples collected in an urban environment in the Negev (southern Israel) were used as a case study. The Negev is located at the margin of the natural dust sources and is frequently subjected to such storms with common duration of several hours to one-day[25,26]. Recent studies relied mainly on satellite images (MODIS) and the HYSPLIT model of air mass backward trajectories to indicate the geographic source of the observed bio samples[1,27–30]. Satellite images of the studied region during the dust storm events (each lasted less than a day), the air mass trajectories and wind directions at the level of the measurement point (consistent with the HYSPLIT trajectories) are displayed in Fig. 1. Both storms were associated with a synoptic system of the (Cyprus) cold low-pressure that typically moves eastward over the Mediterranean Sea[27]. The trajectory of the December air mass (Ev12) extended over North Africa. Due to a deeper cold low in January 2013 (996 mb) than in December 2012 (1004 mb) the origin of the January air mass (Ev13) was attributed to South Europe (Fig. 1).

Physical and chemical properties. As is generally accepted, stronger winds and lower temperatures associated with lower pressure were observed here too (Fig. 1): the levels of major air gas pollutants were not affected by the dust events and remained relatively low as in non-dusty days in the studied region, but those of PM_{10} (particulate matter $\leq$ 10 μm in diameter) in both storms were about 20-fold higher than the background value in the area at non-dusty days (42 μg m^{-3}), as also observed in other strong dust storms in the Negev during the last decade[10]. High levels of PM during dust storm events raise air pollution above the standard values of air quality[10,31]. In arid environments, hourly PM concentrations during dust storms can reach even 10 mg m^{-3} [32].

The particle size distributions of the dust were typically bi-modal for both samples with a cutoff at about 25 μm (Fig. 2). The January sample (Ev13) included higher content of finer particles (45.1%) than that (34.0%) of December's (Ev12) and smaller sizes of the coarser population (peaks at 50 and 60 μm respectively). The possible sources of the finer particles, which can be carried longer distances, are North-African and South-European soils (see mass trajectories and compatible wind directions 10 m above the surface in Fig. 1), whereas the possible proxy source is the Negev soils located close to Be'er Sheva. In both Ev12 and Ev13 samples, there were relatively high ($>$ 22%) frequencies of the class weight of respirable particles ($<$ 10 μm) (Fig. 2) that affect human health[9].

Typical elements were found in both dust samples (Table S1), the most common of which were Si, as in mineral soils, silt and sand fractions ($>$ 50 μm)[33] and Ca originating in sedimentary environments (such as dry lakes), calcareous soils and rocks in the arid land of the Mediterranean basin. The higher percentage of Si in Ev12 (Table S1) may indicate a longer terrestrial transport of the dust (Fig. 1). No significant differences in the contents of Al, Fe, K, Mg and Na were found between the dust samples, but some differences were found in the contents of minor elements: Co was lacking in Ev12; Cu, Os, Ni and Ga were not observed in Ev13.

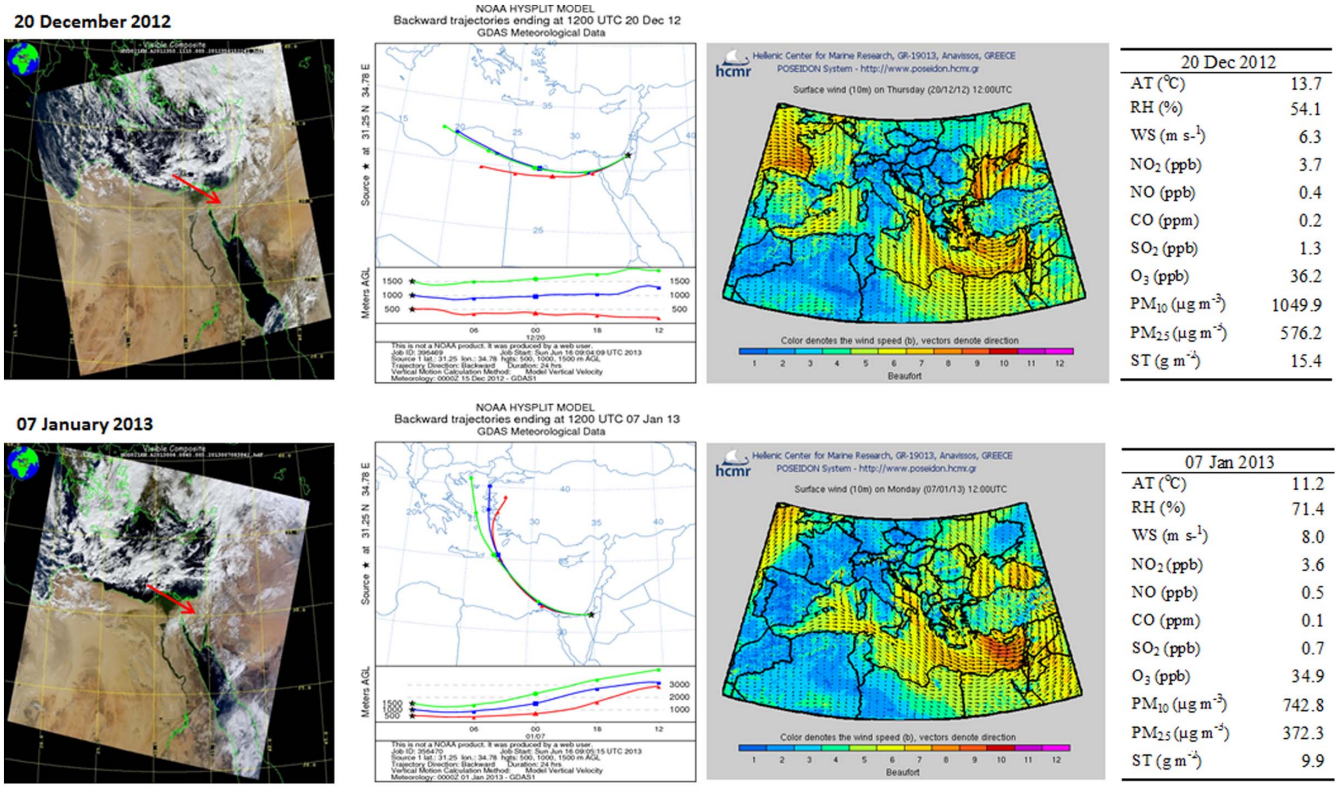
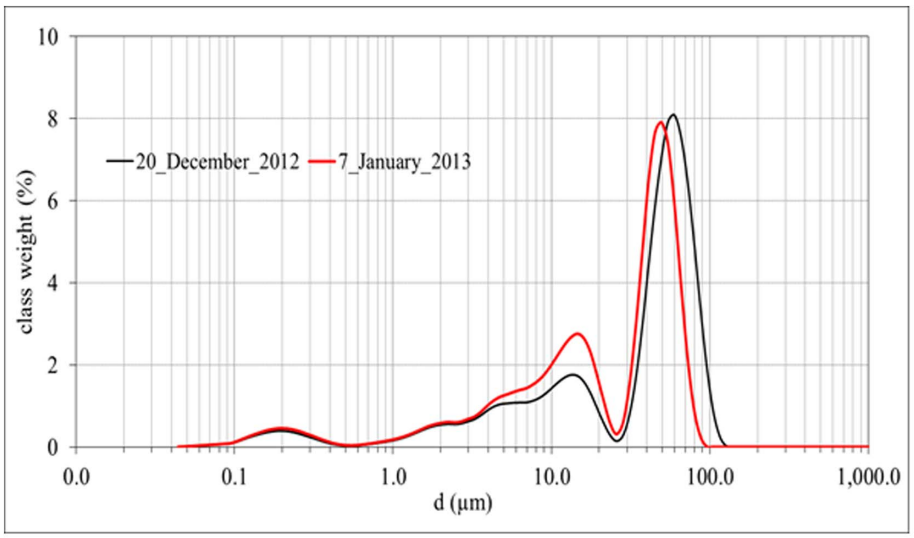


Figure 1 | Satellite images (MODIS) of the studied region during the dust storms along with air mass transport at different heights above ground level (AGL) derived from Backward Trajectories model NOAA/ARL HYSPLIT-4 (credit to: www.noaa.gov) and equivalent wind directions at 10 m above surface level from the HCMR POSEIDON System (credit to: www.poseidon.hcmr.gr). Red arrowheads indicate the sampling site. Right hand side panels: daily recorded averages of major meteorological variables and pollutants. AT – air temperature; RH – relative humidity; WS – wind speed; PM – particulate matter; ST – settled dust.



Basic parameters (μm)	20 December 2012	07 January 2013
Mean	43.7	31.9
Mode 1	62.8	52.1
Mode 2	13.9	15.3
D_{10}	3.3	2.7
D_{50}	48.6	34.5
D_{90}	81.7	63.1
Soil fractions (%)		
Clay (< 2 μm)	7.3	8.4
Fine-silt (2-20 μm)	26.7	36.7
Coarse-silt (20-63 μm)	40.8	47.7
Sand (> 63μm)	25.2	7.2
Aeolian transport (%)		
Short distance (> 70 μm)	20.2	3.2
Medium-distance (20-70 μm)	45.8	51.7
Long-distance (< 20 μm)	34.0	45.1
Respirable particles (%)		
PM_1 (< 1 μm)	4.7	5.6
$PM_{2.5}$ (< 2.5 μm)	8.6	9.8
PM_{10} (<10 μm)	22.9	27.5

Figure 2 | Particle size distribution of the dust samples by a high-resolution laser diffractometer technique (ANALYSETTE 22 MicroTec Plus), with the statistical parameters.

Richness and diversity of organisms in storm dust. The four libraries represent true diversity reasonably well: the average Good's coverage of the reads in both samples (at 97% cut-off) was 94.5% for eukaryotes and 86% for bacteria (Table 1). On the other hand, the relatively low number of observed Operational Taxonomic Units (OTUs; Table 1 and Fig. S1), reaching 46% and 58% saturation (S_{obs}/S_{Chao}; Table 1) for eukaryotes and bacteria respectively, suggests an under-sampling of the dust; diversity may however be biased by tendency of Chao index to overestimate species richness[34]. The bacterial diversity in both samples was significantly higher than the eukaryotic, and the total richness of the dust sample Ev12 was higher than of Ev13 (Table 1).

Venn diagrams (Fig. S2) demonstrate a higher number of bacterial and eukaryotic OTUs that were unique in both dust samples, implying different origins of the biota. The bacterial samples shared 200 OTUs (Fig. S2A), representing the majority of the reads, 54% and 62% for Ev12Bac and Ev13Bac respectively. Most of the unique 1,014 OTUs of Ev12Bac (Table S2) are singletons (i.e., OTU containing a single sequence), implying a relatively low coverage (83%) and high values of richness and diversity of this sample. PCR bias associated with *initial* amplicon generation may impose distortions in the observed community structure, particularly over-estimating rare taxa. Detection limit of the latter may be affected by the approach employed in quality control as well, but pyrosequencing *itself* appears not to impose significant bias of overall community structure estimates[35]. Eukaryotic samples shared only 34 OTUs (Fig. S2B, Tables S3 and S4), which also represented the majority of the sequences, 66% and 47% for Ev12Euk and Ev13Euk. Higher diversity and richness was also observed in Ev12Euk than in Ev13Euk (Table 1).

Prokaryotic sequences (Fig. 3A). Reads of 16S rRNA genes that were retrieved from both dust storm samples belong to the following phyla: *Acidobacteria, Actinobacteria, Bacteroidetes, Deinococcus-Thermus, Firmicutes, Proteobacteria*, *Cyanobacteria*, in addition to unclassified bacteria. Reads of *Gemmatimonadetes* and *Chloroflexi* were found only in Ev12Bac, and *Verrucomicrobia*, only in Ev13Bac. On a class level, the distributions were similar, with the most dominant *Alphaproteobacteria* (44.0% and 46.7% for Ev12Bac and Ev13Bac respectively), *Actinobacteria* (23.9% and 17.5%) and *Betaproteobacteria* (4.0% and 15.5%).

*Actinobacteria***.** This one of the dominant bacterial phylum/class is made up of gram-positive bacteria that are widely distributed in both terrestrial and aquatic ecosystems. Some of the *Actinobacteria* are spore forming which range from motile zoospores to specialized propagules which resist desiccation and mild heat. Most of the sequences are affiliated within the order *Actinomycetales* (95.7% and 99.3% for Ev12Bac and Ev13Bac respectively). The *Geodermatophilaceae* (22.9% and 15.2%), *Micrococcaceae* (22.9% and 42.3%) and unclassified-actinomycetales (27.6% and 23.5%) are the dominated families. The *Actinobacteria* reads belong to 3 major genera, *Kocuria, Arthrobacter* and *Blastococcus*, the species of which are found in various environments such as plants, soils, sediments and rhizomes. Among all bacterial reads, 4.7% in Ev12Bac and 20.5% in Ev13Bac displayed variable similarity (84–100%) to the *Kocuria* species, some with 99% homology to *K. rosea* (KC689298, FJ745378). Several groups are using metagenomic framework to study airborne microbial communities[36,37]. During a severe smog event over Beijing, for example, *Cao et al.*[36] found that the most abundant bacterial phylum, order and species were respectively *Actinobacteria*, *Actinomycetales* and *Geodermatophilus obscurus*, *Modestobacter marinus*, *Blastococcus saxobsidens*, *Kocuria rhizophila*, and *Micrococcus luteus*.

Table 1 | Alpha-diversity indices (97%) based on 454-pyrosequencing data from the dust samples: coverage, sobs (# of OTUs), invSimpson and chao parameters[a]

Sample	N_{seqs}	Good's coverage (%)	S_{obs}	InvSimpson	S_{Chao}	S_{obs}/S_{Chao}
Ev12Euk	3779	93	409	13.87	919.47	0.44
Ev13Euk	3779	96	251	5.18	533.16	0.47
Ev12Bac	4020	83	1,214	58.93	2,142.15	0.57
Ev13Bac	4020	89	869	49.56	1,485.01	0.59

a. N_{seqs} = number of sequences in the sample; S_{obs} = number of observed OTUs; InvSimpson = inversed Simpson's index and S_{Chao} = richness index.

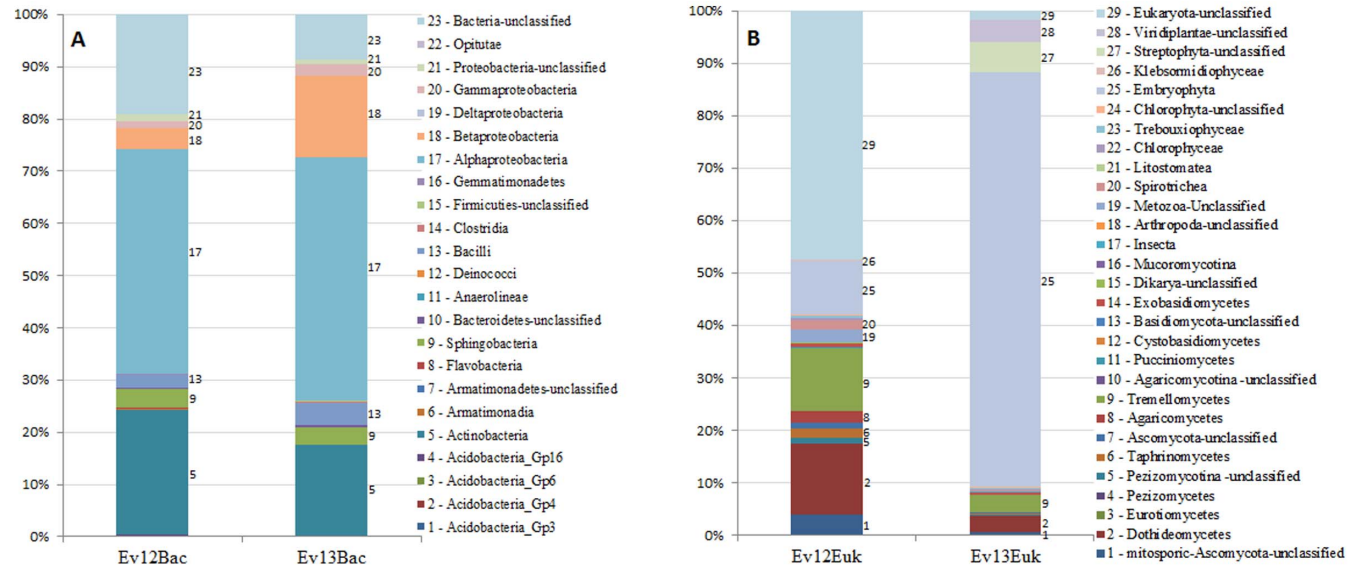


Figure 3 | Distributions of rRNA genes reads, retrieved from the dust samples Ev12 and Ev13. (a) Bacterial 16S reads at the class level. Affiliation of classes to different phyla are: 1–4 *Acidobacteria*; 5 – *Actinobacteria*; 6–7 – *Armatimonadetes*; 8–10 – *Bacteroidetes*; 11 – *Chloroflexi*; 12 – *Deinococcus-Thermus*; 13–15 – *Firmecutes*; 16 – *Gemmatimonadetes*; 17–21 – *Proteobacteria*; 22 – *Verrucomicrobia*; 23 – unclassified. (b) Eukaryotic 18S reads at the kingdom-class levels. Affiliation of taxonomic categories to highest taxonomic ranks are: 1–16 – *Fungi* kingdom (1–7 – *Ascomycota* phylum; 8–14 – *Basidiomycota* phylum; 15 – unclassified *Dikarya* superphylum; 16 – Basal fungal lineages phylum); 17–19 – *Metazoa* kingdom (17–18 – *Arthropoda* phylum; 19 – unclassified *Metazoa*); 20–21 – *Alveolata* superphylum (*Ciliophora* phylum); 22–28 – *Viridiplantae* phylum; 29 – unclassified eukaryota phylum.

Kocuria spp. belonging to *Micrococcaceae* are considered as non-pathogenic commensals that colonize the oropharynx, skin and mucosa, but *K. rosea* is an opportunistic pathogen in immune-compromised patients[38] and *K. kristinae* is associated with acute cholecystitis[39]. In Ev12Bac and Ev13Bac, 11.4% and 14% respectively are closely related to *Arthrobacter* spp. (e.g., HF585203, JX164047, JN680244, AJ576068, AB522428), which are widely distributed in nature, particularly soil. Their overall pathogenic potential is rather low, though some *Arthrobacter* stains were isolated from clinical specimens[40]. Most of the 10.9% and 7.6% of the reads from both samples respectively, affiliated with *Blastococcus* spp., are identical to *B. saxobsidens* (EU977834) and *B. aggregatus* (FR865889), found on rock surfaces in the Mediterranean basin[41]. Very few sequences (11 and 7 respectively) were identical to those of the genus *Microbacterium* found in soil and sewage, some of which have been recognized as pathogens in humans, causing e.g., endophthalmitis[42].

Alphaproteobacteria. This class is a group of gram-negative bacteria that comprise most phototrophic genera, symbionts of plants and animals and a group of pathogens. Orders *Rhodobacterales* (28.9% and 59.5%) and *Rhizobiales* (44.6% and 17.1%), are among the largest revealed in both Ev12Bac and Ev13Bac respectively; the former are dominant and ubiquitous primary surface colonizers in temperate coastal waters of the world[43] and the latter include nitrogen fixing symbionts and pathogenic to animals and plants[44]. *Alphaproteobacteria* in both samples showed similar distribution on a family and genus levels and was dominated by the following genera: *Paracoccus* (5.3% and 15.8%), *Rubellimicrobium* (15.5% and 24.5%) and *Skermanella* (6.7% and 9.3%). Some of the ubiquitous spp. of the genus *Brevundimonas* are considered to be opportunistic pathogens in immune-compromised hosts[45], e.g., *B. vesicularis* and *B. diminuta* are frequently isolated species in human infections. Only 16 and 14 reads here were identical to *B. vesicularis* (KC494336, GU430201, HM755555).

Betaproteobacteria. This is the 3rd most dominant class, with 89% and 83% of the reads for Ev12Bac and Ev13Bac respectively, belonging to the order *Burkholderiales* that includes several pathogenic genera e.g., *Burkholderia* and *Bordetella*, but none of these reads belongs to them. Among the reads of this order, 43.6% and 62.7% are identical to *Massilia yuzhufengensis* (JQ409016) isolated from ice drilled in Yuzhufeng Glacier, Tibetan Plateau, China[46].

Unclassified bacteria. A large portion of these reads in Ev12Bac and Ev13Bac (19.1% and 8.6% respectively) were revealed by the RDP database; when analyzed against NCBI GenBank, 56.5% and 11.9% of these were identical to the cyanobacterial species *Oscillatoria nigroviridis* PCC 7112 (CP003614).

Viability of pathogenic microorganisms. Viability is a meaningful parameter in developing predictive models for disease dispersal[29]. An average of about 70% viable bacteria has recently been estimated by fluorescent staining in anticyclone air[28], but the majority (>99%) of microorganisms from the environment resist cultivation in the laboratory[36,47], hence culture dependent methods cannot be used to assess viability of the pathogens in dust samples.

Eukaryotic sequences (Fig. 3B). *Dothideomycetes*. This is the largest and most diverse class of ascomycetes that includes several plant pathogens (e.g., *Phaeosphaeria nodorum, Venturia inaequalis*). Ev12Euk and Ev13Euk contained 13.5% and 3.15% such reads respectively, most of them (8.9% and 1.5%) are highly similar (99–100%) to *Cladosporium* spp. (e.g. JX273066, JN974018, JN546118), some of which cause infections of the skin and toenails, sinusitis and pulmonary infections. Their airborne spores are significant allergens[48] and in large amounts they can severely affect asthmatics[49]. Dominant sequences retrieved from indoor dust of urban area in central Finland were identical to *C. cladosporioides* and *C. herbarum*[23]. The rest of the *Dothideomycetes* reads (4.3% and 1.3%) were identical to *Alternaria* spp. (e.g., KC584600, KC584596, KC584599), major plant pathogens and common humans allergens[48], causing hay fever or hypersensitivity reactions that sometimes lead to asthma[49]. Many health disorders are caused by these fungi, which grow on skin, mucous membranes, the eyeballs and the respiratory tract[50]. *Alternaria* and *Epicoccum* spp. (producing multicellular dictyospores > 10 μm) are abundant allergenic fungi, mostly in the larger particle-

size ranges ($d_a > 44.7$ μm), whereas *Cladosporium* is an abundant allergenic fungal genus, distributed evenly across all the particle-sizes ranges[51]. Viable microbial populations, including presumptive plant pathogens *Alternaria infectoria* and *Chaetomium globosum*, have recently been detected in Asian air samples even after traveling 10 days across the Pacific Ocean in the free troposphere, information that has significant implications for epidemiology[29].

Tremellomycetes. This fugal class (*Agaricomycotina, Basidiomycota*) is a dimorphic, nutritionally heterogeneous group comprising of saprotrophs, animal parasites, severe human pathogens and fungicolous species[52]. Ev12Euk and Ev13Euk contain 12% and 3.3% of such reads that are highly similar to *Cryptococcus* spp. and *Filobasidium* spp.; about 60% of them are identical to *C. albidus* (HQ231895) that occasionally causes moderate-to-severe diseases, specifically meningitis, in patients with compromised immunity[53].

Mitosporic Ascomycota. These fungi comprise a heterogeneous group and represent more than half of *Ascomycota* lacking a sexual state; many pathogenic fungi in plants and mammals, including humans, belong to this group[54]. Ev12Euk and Ev13Euk contain 3.9% and 0.5% reads respectively belonging to this group.

Embryophyta. This is the most familiar subkingdom of green plants (*Viridiplantae*), informally called land plants, excluding green algae[55], and was represented by 10.4% and 79.2% in Ev12Euk and Ev13Euk respectively. This high percent of plant reads in the latter is consistent with southern Europe as the origin of the air mass in January 2013. Among them, 3.6% and 2.7% in Ev12Euk and Ev13Euk respectively displayed 98–100% homology to leafy trees from the *Morus* spp. (GU476477 and L24398), *Moringa oleifera* (U42786), *Plocosperma buxifolium* (HQ384684), *Metteniusa tessmanniana* (AM421127) and *Olea europaea* (L49289). Additional 3% of *Embryophyta* reads in each Ev12Euk and Ev13Euk are identical to *Bryum pseudotriquetrum* (KC291525), *Blindia acuta* (AF023681) and *Pottia truncata* (X95935). Among the *Embryophyta* reads, 2.3% and 40.4% in Ev12Euk and Ev13Euk respectively are highly similar (98–99%) to *Cratylia* sp. (JX158808) and chickpea *Cicer arietinum* (AHII01138308) that belong to *Fabaceae* commonly known as legume, the 3rd-largest land plant family and economically important[56]. Dust events that transport pollen long-distances introduce vegetation changes and are prone to errors in studies (paleoecology) that interpret past local vegetation based on presence of pollen[11].

Sequences affiliated with class *Pinopsida* (mostly conifers) were the 2nd largest group (25.4%) of the *Embryophyta* in Ev13Euk, none of them were found in Ev12Euk. This group included reads highly similar (99–100%) to e.g., *Cupressus gigantea* (EF053166), *Tetraclinis articulata* (EU161293), *Juniperus morrisonicola* (EF673744), *Chamaecyparis pisifera* (EF053165), *Picea morrisonicola* (AB026939), *Pinus luchuensis* (D38246). Finally, flowers and grass also appeared only in Ev13Euk as 2.8% of the *Embryophyta* reads that are similar (97–100%) to *Arabidopsis thaliana* (X16077), *Arctium lappa* (JF703098), *Tagetes* sp. *Nickrent* 3061 (U42501), *Sinapis alba* (X17062), *Lolium multiflorum* (AY846367), *Festuca rubra* (AF168844), and other species. Air masses originating in southern Europe do not usually harbor particles but include primary biological aerosol consisting of viruses, bacteria, fungal spores and plant pollen. The January dust storm here may have collected the particles in the Sinai desert on its way to the Negev.

Alveolata. This superphylum is a monophyletic group of primarily single-celled eukaryotes that have adopted extremely diverse modes of nutrition such as predation, photoautotrophy and intracellular parasitism. Most alveolates fall into 3 main subgroups: ciliates, dinoflagellates and apicomplexans. Ciliates are one of the most important groups of protists common to water tarns and soils, and have many ecto- and endosymbiotic members, as well as some obligate and opportunistic parasites. Both dust samples (Ev12Euk and Ev13Euk) encompass 1.7% and 0.1% respectively of such reads; most reads from the former are identical to *Halteria* spp. with typical size of 20–50 μm and predominantly found in fresh water habitats.

Arthropoda. Members of this phylum are exoskeleton-containing invertebrates with a segmented body and jointed appendages, and include insects, arachnids, crustaceans and myriapods. Ev12Euk contained 2.6% reads, most of which are affiliated with unclassified Metazoa/Animalia (with similarity of ≤93% in the BLAST database) and some belong to infraclass Neoptera—a group that includes most of the winged insects. Ev13Euk included only 0.26% such sequences.

Unclassified eukarya. According to SILVA database classification, reads from Ev12Euk and Ev13Euk include respectively 47.5% and 1.8% unclassified eukaryotes; they were therefore further analyzed using the BlastN program: about half (23%) of the Ev12Euk reads were identical to *Rhizophlyctis rosea* (NG017175), a soil cellulose-decomposing zoospores-producing fungus[57]. Additional 4.5% were identical to other ubiquitous zoospore-producing soil-dwelling fungal species belonging to *Gaertneriomyces, Spizellomyces, Powellomyces* and other genera (e.g., JN020240, GU568157, JN940943, FJ827646, AY349038, FJ827660, HQ901755, HQ901759). Additional 7.3% of Ev12Euk unclassified reads were identical to sequences of nematodes such as *Aphelenchus avenae* (AY284640 and AB731165), which is mycophagous and capable of withstanding droughts[58]. No nematodes were observed in Ev13Euk. The remaining (12.7%) of unclassified eukaryotic reads from Ev12Euk appeared as singletons with low similarity to known sequences. Unclassified Ev13Euk reads include only 69 sequences, 9 of which showed high similarity (97%) to an uncultured ciliate clone QD09 (HQ909037).

Concluding remarks. The results demonstrate that the diversity of organisms in airborne dust is higher than previously reported and may rival that of the other (terrestrial or aquatic) environments.[11,12] Dust of Ev13, of south European origin, was rich with reads affiliated to *Betaproteobacteria*, and to *Embryophyta* (land plants) in general, particularly conifers. On the other hand, dust of Ev12 (of north African origin) contained significantly more *Actinobacteria*, fungi, unclassified bacteria and eukaryotic sequences. Reads affiliated to allergenic and pathogenic species existed in both airborne dust samples. Intercontinental aerobiology studies will aid in developing predictive models for disease dispersal[29]. Despite its limitations[47], culturing data may be valuable by knowing what species remain viable after long distance atmospheric transport[30].

This study will likely affect the methodology to analyze climatic factors, soil sources, levels of particulate matter and air biology associated with dust storms, and the assessment of the possible impacts on the ecosystems and hazards to public health.

Methods

Dust sample collection and physico-chemical measurements. The samples of outdoor dust were collected in Be'er Sheva (Israel) on a building roof within the campus of Ben-Gurion University of the Negev, during storm events that occurred in the "dust season" (October - May): on December 20, 2012 (Ev12) and on January 7, 2013 (Ev13). Both events were typically strong storms in the studied area. The spatial extent of each event was recorded by satellite images (MODIS Level 1 and VIIRS Level 1) (Fig. 1). The air mass transport in the region was detected through Backward Trajectories model (NOAA/ARL HYSPLIT-4)[59] prior and during the events. The wind directions at 10 m level were retrieved by the POSEIDON System. The following major pollutants and meteorological variables were recorded: NO_2 (ppb), NO (ppb), CO (ppm), SO_2 (ppb), O_3 (ppb), wind speed ($m\,s^{-1}$), air temperature (°C), and relative humidity (%). Atmospheric concentrations ($\mu g\,m^{-3}$) of PM_{10} and $PM_{2.5}$ and total settled dust ($g\,m^{-2}$) were recorded simultaneously during the storms in a monitoring system nearby the dust samplers.

The sterilized settling-dust collectors consist of a rectangular plastic tray (40 × 25 × 10 cm) filled with layers of glass/quartz marbles (10 mm in diameter). Atmospheric dust particles that cross the tray aperture are trapped in the marble matrix due to the matrix cohesion and roughness along with reduced air velocity near the surface of the tray. At the end of each event the dust samples were moved into sterilized glass vials for size distribution and elemental analyses. Size distributions of particles over the range of 0.08 to 2000 μm were obtained by a high-resolution laser

diffractometer (ANALYSETTE 22 MicroTec Plus). Each sample was dispersed by sonication (at 38 kHz) in a Na-hexametaphosphate solution (0.5%), then transferred to a fluid module of the instrument (containing deionized water), and subjected to 3 consecutive 1 min runs at a medium pump speed of 6 L min^{-1}. The data were processed using the Mie scattering model (RI = 1.56, AC = 0.01) with an error < 5.0%. The MasControl software was employed to determine the following relevant parameters: mean size, median, modes in multiple modal distributions, sorting values, size fraction weights.

Elemental analyses were performed by X-Ray Fluorescence (XRF) method using XRF Spectrometer, Panalytical Co., model Axios [WDXRF (wavelength dispersive), 1 kW]. Omnian software was used for quantitative analysis.

DNA extraction, PCR amplification and pyrosequencing. Total DNA was extracted from 0.5 g of the 2 dust samples (Ev12 and Ev13) using a Power Soil Isolation Kit (MoBio Laboratories, Carlsbad, CA) with the bead-beating protocol supplied by the manufacturer, and quality and concentration were assessed using a NanoDrop ND 1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE).

Low concentration (~5 ng μl^{-1}) of the total genomic DNA of each sample was amplified using a Biometra TGradient thermocycler (Biometra, Gottingen, Germany) and 2 pairs of primers: the universal bacterial primers 8F, AGAGTTTGATYM-TGGCTCAG and 907R, CCGTCAATTCMTTTGAGTTT to amplify a ~900 bp region of the 16S rRNA genes, and the universal eukaryotic primers NSF370/18, AGGGYTCGAYYCCGGAGA and NSR1787/18, CYGCAGGTTCACCTACRG targeting 18S rRNA genes amplifying the ~1400 bp. The reaction mixture included 12.5 µl ReddyMix (ABgene, Surrey, UK), 1 µl of 10 µM concentrations of each primer (forward and reverse), 1 µl of 25 mM bovine serum albumin, 1–3 µl of the sample genomic DNA (10–40 ng µl−1), and water for a total volume to 25 µl. An initial denaturation hot start of 4 min at 95°C was followed by 30 cycles of the following incubation pattern: 94°C for 30 s, 54°C for 30 s, and 72°C for 1–1.5 min.

The amplicons were submitted to Molecular Research laboratory (MR DNA, Shallowater, TX) for PCR optimization and pyrosequencing analyses utilized Roche 454 (454 Life Sciences, Branford, CT). The company's primer 27Fmod AGRGTTTGATCMTGGCTCAG was used to obtain bacterial reads of the hypervariable regions V1-V2 from the dust samples (Ev12 and Ev13), designated Ev12Bac and Ev13Bac, and the primer Euk528F CCGCGGTAATTCCAGCTC was used for the V4 eukaryotic reads designated Ev12Euk and Ev13Euk.

Analysis of organismic diversity. In total, 15,019 bacterial and 15,424 eukaryotic sequence reads were recovered and subjected to MOTHUR software version 1.9.1[60], leaving 4020 bacterial reads for each sample, Ev12Bac and Ev13Bac, and 3779 eukaryotic reads for each Ev12Euk and Ev13Euk, for further considerations.

The sequences were trimmed using MOTHUR software for removal of primers, barcodes, ambiguous nucleotides, long homo-polymers, reads below a minimum quality score of 25 and sequences shorter than 150 bases. Sequences were aligned, checked for chimeras, filtered, and classified using the Ribosomal Database Project II website (RDP Release 10) for bacteria (Table S2) and SILVA reference files for eukarya (Tables S3 and S4). Reads classified as Chloroplast, Mitochondria or "unknown" were also removed. The sub.sample function in MOTHUR, used to randomly select equally-sized reads from each library, further reduced data sets for subsequent analyses. The pyrosequencing population data were also analyzed by multiple sequence alignments using the dist.seqs function[60].

MOTHUR operational taxonomic units (OTUs) analyses were conducted (by a 0.03 distance level) to present α-diversity: Chao1 richness estimators, the inverse Simpson diversity index and rarefaction curves at 0.03 distances, which were plotted on a line chart using Microsoft Excel. PC-ORD 6 software (MJM Software Design, OR) was used to calculate beta-diversity.

Raw sequencing data was deposited in the MG-RAST (metagenomics.anl.gov) archive under accession numbers: 4534886.3 for Ev12Bac; 4534887.3 for Ev12Euk; 4534888.3 for Ev13Bac; 4534889.3 for Ev13Euk.

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Acknowledgments

This investigation was partially supported by grants from the Israeli Environment and Health Fund (No. RGA1004) and the Israel Science Foundation (1100/11), both to I.K. and from the Ramat Hovav Council (to A.K.). We thank the reviewers for their helpful insight and comments that improved the manuscript.

Author contributions

I.K., A.Z., E.B.D. and A.K. carried out experimental design; I.K. and H.K. carried out physical-chemical and transport measurements; E.B.D. performed DNA isolation and amplification; L.A. carried out 16S rRNA data analyses; E.B.D. and I.K. coordinated the project; all authors contributed to manuscript preparations, discussed the results and implications and commented on the manuscript.

Additional information

Supplementary information accompanies this paper at http://www.nature.com/scientificreports

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Katra, I. *et al.* Richness and Diversity in Dust Stormborne Biomes at the Southeast Mediterranean. *Sci. Rep.* **4**, 5265; DOI:10.1038/srep05265 (2014).