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<td>Katra, I., Arotsker, L., Krasnov, H., Zaritsky, A., Kushmaro, A., &amp; Ben-Dov, E. (2014). Richness and Diversity in Dust Stormborne Biomes at the Southeast Mediterranean. Scientific Reports, 4, 5265-.</td>
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<td><strong>Date</strong></td>
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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 aerosols1, 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 year2. Climatic variations affect the land surfaces of aeolian (wind-driven) systems3, and droughts have significantly contributed to increased dust emissions4,5. The contribution of anthropogenic soils, e.g. agricultural fields and industrial yards, are of increasing concern6. Up to 50% of the total atmospheric dust originates from disturbed soils7, which may contain different particle compositions from that of natural dust. The windblown dust can travel tens of thousands of kilometers before being deposited8, depending on the particle characteristics (size, chemistry) and the air-mass properties (e.g., velocity, density, height)9. The size and chemical compositions vary in space and time and may determine the potential impact on air quality and human health9,10.

Airborne dust particles contain different microorganisms that are exceedingly mobile in space and time11,12. 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 0.10513,14. Dust storms originating in the Saharan desert heavily affect the south-eastern Mediterranean basin, mostly during the winter and spring15; 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 regions11,12,18. 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 dust19–24, 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-day25,26. 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 samples1,27–30. 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 Sea27. 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 $\leq 10 \mu m$ in diameter) in both storms were about 20-fold higher than the background value in the area at non-dusty days (42 $\mu g \ m^{-3}$), as also observed in other strong dust storms in the Negev during the last decade10. High levels of PM during dust storm events raise air pollution above the standard values of air quality10,31. In arid environments, hourly PM concentrations during dust storms can reach even 10 mg $m^{-3}$.

The particle size distributions of the dust were typically bi-modal for both samples with a cutoff at about 25 $\mu m$ (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 $\mu m$ 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 $\mu m$) (Fig. 2) that affect human health9.

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 $\mu m$)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.
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 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. 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. 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. During a severe smog event over Beijing, for example, Cao et al. 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>
<thead>
<tr>
<th>Sample</th>
<th>N_{seq}</th>
<th>Good’s coverage (%)</th>
<th>S_{obs}</th>
<th>InvSimpson</th>
<th>S_{Chao}</th>
<th>S_{obs}/S_{Chao}</th>
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<tr>
<td>Ev12Euk</td>
<td>3779</td>
<td>93</td>
<td>409</td>
<td>13.87</td>
<td>919.47</td>
<td>0.44</td>
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<tr>
<td>Ev13Euk</td>
<td>3779</td>
<td>96</td>
<td>251</td>
<td>5.18</td>
<td>533.16</td>
<td>0.47</td>
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<tr>
<td>Ev12Bac</td>
<td>4020</td>
<td>83</td>
<td>1,214</td>
<td>58.93</td>
<td>2,142.15</td>
<td>0.57</td>
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<tr>
<td>Ev13Bac</td>
<td>4020</td>
<td>89</td>
<td>869</td>
<td>49.56</td>
<td>1,485.01</td>
<td>0.59</td>
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α. N_{seq} = number of sequences in the sample; S_{obs} = number of observed OTUs; InvSimpson = inversed Simpson’s index and S_{Chao} = richness index.
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 and K. kristinae is associated with acute cholecystitis. In Ev12Bac and Ev13Bac, 11.4% and 14% respectively are closely related to Arthrobacter spp. (e.g., HF585203, JX164047, JN608244, 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. Most of the 10.9% and 7.6% of the reads from both samples respectively, affiliated with Blastococcus spp., are 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.

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 and the latter include nitrogen fixing symbionts and pathogenic to animals and plants. 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, 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 Yuzhu Feng Glacier, Tibetan Plateau, China.

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. An average of about 70% viable bacteria has recently been estimated by fluorescent staining in anticyclone air, but the majority (>99%) of microorganisms from the environment resist cultivation in the laboratory, 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 and in large amounts they can severely affect asthmatics. Dominant sequences retrieved from indoor dust of urban area in central Finland were identical to C. cladosporioides and C. herbarum. 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, causing hay fever or hypersensitivity reactions that sometimes lead to asthma. Many health disorders are caused by these fungi, which grow on skin, mucous membranes, the eyeballs and the respiratory tract. Alternaria and Epicoccum spp. (producing multicellular dictyospores > 10 μm) are abundant allergenic fungi, mostly in the larger particle-
size ranges (dₜ > 44.7 μm), whereas Cladosporium is an abundant allergenic fungal genus, distributed evenly across all the particle-sizes ranges²⁹. 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 epidemiology²⁹.

Tremellomyces. This fungal class (Agaricomycotina, Basidimycota) is a dimorphic, nutritionally heterogeneous group comprising of saprotrophs, animal parasites, severe human pathogens and fungi-colous species³². 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. albidas (HQ231895) that occasionally causes moderate-to-severe diseases, specifically meningitis, in patients with compromised immunity³⁶.

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 group³⁵. 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 algae³⁶, 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), Morina oleifera (U42786), Plosoperma buxifolium (HQ384684), Metteniusa tesselmanniana (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 Potti truncata (X95935). Among the Embryophyta reads, 2.3% and 40.4% in Ev12Euk and Ev13Euk respectively are highly similar (98–99%) to Cratylia spp. (IX158808) and chickpea Cicер arietinum (AHII01138308) that belong to Fabaceae commonly known as legume, the 3rd-largest land plant family and economically important³⁶. 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 pollen³⁶.

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), Tetracis 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 plants but include primary biological aerosol consisting of viruses, bacteria, fungal spores and plant pollen. The January dust storm here may have collected the plants 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 algaeevolve into more complex organisms (e.g., JN020240, GU568157, JN940943, FJ827646, AY349038, FJ827667, HQ901755, HQ901759). Additional 7.3% of Ev12Euk unclassified eukaryotic reads were identical to sequences of nematodes such as Aphelechus avenue (AY284640 and AB731165), which is mycophasal and capable of withstanding droughts³⁷. 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 (HQ900937).

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.³⁸,³¹ 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 microbiology studies will aid in developing predictive models for disease dispersal³⁹. Despite its limitations⁴⁰, culturing data may be valuable by knowing what species remain viable after long distance atmospheric transport⁴⁰.

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 2) (Fig. 1). The air mass transport in the region was detected through Backward Trajectories model (NOAA/ARL HYSPIT-4)² 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: NO₂ (ppb), NO (ppb), CO (ppm), SO₂ (ppb), O₃ (ppb), wind speed (m s⁻¹), air temperature (° C), and relative humidity (%). Atmospheric concentrations (μg m⁻³) of PM₁₀ and PM₂.₅ 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
41. Urzi, C., Salamone, P., Schumann, P., Rohde, M. & Stackebrandt, E. Blastococcus saxoidoxis sp. nov., and emended descriptions of the genus Blastococcus Ehrens


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/srep.

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).

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