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ORIGINAL ARTICLE

Microbial hitchhikers on intercontinental dust: catching a lift in Chad

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Ancient mariners knew that dust whipped up from deserts by strong winds travelled long distances, including over oceans. Satellite remote sensing revealed major dust sources across the Sahara. Indeed, the Bodélé Depression in the Republic of Chad has been called the dustiest place on earth. We analysed desert sand from various locations in Chad and dust that had blown to the Cape Verde Islands. High throughput sequencing techniques combined with classical microbiological methods showed that the samples contained a large variety of microbes well adapted to the harsh desert conditions. The most abundant bacterial groupings in four different phyla included: (a) Firmicutes—Bacillaceae, (b) Actinobacteria—Geodermatophilaceae, Nocardiodaceae and Solirubrobacteraceae, (c) Proteobacteria—Oxalobacteraceae, Rhizobiales and Sphingomonadaceae, and (d) Bacteroidetes—Cytophagaceae. Ascomycota was the overwhelmingly dominant fungal group followed by Basidiomycota and traces of Chytridiomycota, Microsporidia and Glomeromycota. Two freshwater algae (Trebouxiophyceae) were isolated. Most predominant taxa are widely distributed land inhabitants that are common in soil and on the surfaces of plants. Examples include *Bradyrhizobium* spp. that nodulate and fix nitrogen in *Acacia* species, the predominant trees of the Sahara as well as *Herbaspirillum* (Oxalobacteraceae), a group of chemoorganotrophic free-living soil inhabitants that fix nitrogen in association with Gramineae roots. Few pathogenic strains were found, suggesting that African dust is not a large threat to public health.
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Introduction

Deserts are the major source of air-borne dust (Prospero *et al.*, 2005; Collaud Coen *et al.*, 2003; see also Gorbushina *et al.*, 2007). 1000—3000 $\times 10^6$ tonnes (Mt) of desert soil (convergence value ~2000 Mt—Shao, 2008) travel long distances on winds each year (Caquineau *et al.*, 1998; Shao *et al.*, 2011). Of this total, 130—760 Mt originate from the Sahara (for example, Alfaro *et al.*, 1998; Callot *et al.*, 2000; Middleton and Goudie, 2001 and Shao, 2008). About three-quarters of this dust falls back to land in Africa: most of the rest into oceans. By analysing Total Ozone Mapping Spectrometer data, Middleton and Goudie (2001) and Goudie and Middleton,

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(2001, 2006) showed that the major African dust sources include southern Algeria, western Mali, eastern Mauritania and the Bodélé Depression. The latter is located at the southern edge of the Sahara Desert, is the lowest point in Chad, was the floor of a much larger Mega-Lake Chad and has been called 'the dustiest place on earth' (Giles, 2005; Engelstaedter *et al*., 2006; Warren *et al*., 2007). Winds that sweep between the Tibesti and the Ennedi mountains in northern Chad are funnelled across this depression.

Over the past 10 000 years, northern Africa has changed from 'green' to a hot desert (Kröpelin *et al*, 2008). Persistent droughts, coupled with increased demand for irrigation, reduced Lake Chad to about five per cent of its original area (Drake and Bristow, 2006). As Mega-Lake Chad receded to the current position of Lake Chad, diatoms sedimented from the fresh water and now form the surface crust. The silts and sediments dried on the lake bed and it is from here that the coupled saltation/sand blasting processes heave crust particles into the air. During the northern winter, the depression produces an average of 700 000 tonnes dust/day (Koren *et al*., 2006).

Dust storms over the Atlantic are part of seafaring lore. Charles Darwin and his friend Christian Ehrenberg showed that airborne dust contains organic material (Darwin, 1846). Microbes that hitchhike on desert dust are now known to spread across the globe (Griffin *et al*., 2003a, b; Kellog *et al*., 2004; Prospero *et al*., 2005; Kellogg and Griffin, 2006; Weir-Brush *et al*., 2004; Lim *et al*., 2011). Gorbushina *et al.* (2007) used geochemical methods to show that the Darwin/Ehrenberg dust probably originated from the Sahara.

As microbes need a dust-like vehicle to travel long distances (Yamaguchi *et al*., 2012), those microorganisms that are present in desert soils at the source of dust events are blown into the stratosphere and across continents. Surface soils of the deserts of the Republic of Chad (afterwards Chad) are an obvious place to study microbes before they begin intercontinental travel. Sand samples from Bardaï in the desert zone to the northwest of the country and others from the arid Sahelian belt in the east (Bahaï Wadii, Oure Cassonia, Vers Ourba and Vers Iriba) were collected by the International Committee of the Red Cross. One of the authors (AC) provided dust samples from the Bodélé Depression (Warren *et al*., 2007 and Chappell *et al*., 2008), whereas AAG, JP and WJB collected dust during a remake of Darwin's Beagle voyage in 2009. Here we catalogue the microbiological contents of these samples using classic techniques, metagenomic methods and high throughput sequencing (HtS) specially developed to reveal uncultivable microorganisms that adhere to dust (Giongo *et al*., 2012).

Materials and methods

The samples

Three sets of samples were analysed: **I** sand from various regions of Chad (Table 1); **II** dust collected from the Bodélé Depression in Chad (Table 2) and **III** dust harvested on board the 'Stad Amsterdam' (Table 3)(Figure 1). Isolation of algae, bacteria and fungi, the extraction of DNA from sand/dust, DNA sequencing, geochemical analyses as well as scanning electron microscopy were performed exactly as described in Giongo *et al.* (2012). Nitrifying bacteria were isolated according to MacFarlane and Herbert (1984). Since samples **II** were restricted in size and samples **III** were vanishingly small, we only sought cultivable microbes and performed partial geochemical analyses on them.

Statistical analyses

To test whether the distributions of microbes were affected by geographic and/or geochemical factors, the presence of the 40 most abundant families were transformed ($\log_{10}$), Spearman correlation matrices created (alpha = 0.01) and principal component

Table 1 Coordinates and description of the sand collected from Chad

Location	*Collector*[a]	*Date collected*	*Latitude*	*Longitude*	*Altitude (m)*	*Description*
C1 Bardaï	CICR	2007	N21.21.999	E17.03.202	1105	Large, multi-coloured grains 2–3 mm
C2 Bardaï	CICR	2007	N21.21.999	E17.03.202	1105	Fine, red sand
C3 Bahaï Wadi I	Sandman B	30.04.07	N15.37.473	E23.00.626	781	Clumped, red-brown sand
C4 Bahaï Wadi II	Sandman C	30.04.07	N15.37.473	E23.00.626	781	Fine, ochre-coloured, flowing sand
C5 Oure Cassonia	Sandman A	30.04.07	N15.40.215	E23.02.247	797	Fine 1–2 mm, red sand
C6 near Ourba	Sandman D2	01.05.07	N15.23.905	E22.42.297	836	Fine 1–2 mm, red sand
C7 near Iriba	Sandman G	01.05.07	N15.20.080	E22.24.634	898	Fine 1–2 mm, red sand, with large, white grains
C8 near Ourba	Sandman D1	01.05.07	N15.23.905	E22.42.297	836	Fine 1–2 mm, red sand, some clumps
C9 near Ourba	Sandman F	01.05.07	N15.23.648	E22.30.953	895	Fine 1–2 mm, red sand, with large, white grains

where: altitude is above sea level.
[a]Sampling was organised by Dr. Agathe Stricker of the Comité international de la Croix Rouge (CICR), Genève, Switzerland from various locations in the Republic of Chad. As the actual collectors wish to remain anonymous, they have been listed either as CICR or Sandman the '*nom de plume*' that the collectors themselves suggested. C1 and C2 were collected in Bardaï, a small oasis town (and garrison) in the Bourkou-Ennedi-Tibesti Region near the Tibesti Mountains (a group of largely inactive volcanoes) in the Central Saharan Desert. All other samples were collected in Eastern Chad. C4 was from Bahaï-Wadi II near Iriba, a town in the Wadi Fira Region. Samples C6, C8 and C9 were taken near Ourba a gathering place close to the city of Biltine, the capital of the Wadi Fira region. Sample C7 was from a refugee camp near Iriba, the main town in the Department of Kobé in the region of Wadi Fira. All samples were aseptically scraped directly in 50 ml Falcon tubes for storage and transport.

Table 2 Dust collected during the BodEx expedition at different heights above the desert surface

Sample #	*Collector*	*Date collected*	*Latitude*	*Longitude*	*Altitude (asl) & height*	*Description*
Wp 44 Bodélé near Chica	see Chappell *et al.* (2008)	10.03.05 to 12.03.05	N16.52.53	E18.32.548	179 m	surface
Wp 45 Bodélé near Chica	see Chappell *et al.* (2008)	10.03.05 to 12.03.05	N16.52.53	E18.32.548	179 m 0.75 m	dust bottom
Wp 58 Bodélé near Chica	see Chappell *et al.* (2008)	10.03.05 to 12.03.05	N16.58.5353	E18.28.036	179 m 2.4 m	dust top

where: altitude is above sea level and height above the desert surface. The multi-disciplinary Bodélé Field Experiment (BoDEx 2005—http://www.geog.ox.ac.uk/research/climate/projects/bodex/index.html.) took place from February 28 to March 13, 2005 at a site called 'Chicha' (16° 53' N, 18° 33' E; 179 m asl) at the eastern margin of a large diatomite deposit that originated from Mega-Lake Chad (see Warren *et al.*, 2007; Chappell *et al.*, 2008). Samples were collected in a narrow corridor of desert pavement on the eastern edge of the sand sea of the Erg d'Djourab and the far eastern margin of the massive diatomite surface of paleo Lake Chad (16° 52' 53" N 18° 32' 54.8" E and 16° 58' 53" N 18° 28' 03.6" E). One of the authors (AC) took part in the BodEx expedition and provided three samples—a 'grab' sample of the surface material comprising mobile sediment overlying the diatomite playa (Wp 44), dust collected at 0.75 m above the surface during a dust storm (Wp 45) and dust collected at 2.4 m above the desert surface during the same storm (Wp 58). At the time of analysis, the samples had been stored for three years and had been X-rayed twice by custom officials during transportation from the Republic of Chad to the UK and from the UK to Switzerland.

Table 3 Dust samples collected on board the 'Stad Amsterdam'

Sample	*Location*	*Place*	*Date*
Ft1 to Ft4	Ilha de Santiago, leaving port 14° 07' 13" N 23° 43' 65" W	Mizzen mast, ≈20 m asl. Ft1 – P, Ft2 – A; Ft3 – F; Ft4 – S*.	22.09–23.09.2009
Ft5 to Ft8	10° 55' 10" N 25° 18' 2" W	Mainmast, ≈20 m asl. Ft5 – SA, Ft6 – SF, Ft7 – PA, Ft8 – PF.	23.09–25.09.2009
Alu M	10° 55' 10" N 25° 18' 2" W	Mainmast	23.09–25.09.2009
Alu A	Ilha de Santiago, leaving port 14° 07' 13" N 23° 43' 65" W	Toilet roof (starboard/windward side)	22.09–23.09.2009
Alu2 (Control)	At sea between Arquipélago de São Pedro e São Paulo 00°55′1″N 29°20′7″W & Fernando de Noronha 3° 51′ S, 32° 25′ W	Toilet roof (port/leeward side)	26.09–27.09.2009
Alu B	Ilha de Santiago, leaving port 14° 07' 13" N 23° 43' 65" W	Toilet roof (port/leeward side)	22.09 - 23.09.2009
Alu4 (Control)	At sea between Arquipélago de São Pedro e São Paulo 00°55′1″N 29°20′7″W & Fernando de Noronha 3° 51′ S, 32° 25′ W	Toilet roof (starboard/windward side)	26.09–27.09.2009

Abbreviations: A,aft; Alu,aluminium foil; F,fore; Ft,Falcon tubes; P,port side; S,starboard side. *—dust visible to the naked eye.
The Public Dutch Television Network VPRO (VPRO Beagle, Postbus 11, 1200 JC Hilversum, The Netherlands—http://www.vpro.nl) chartered the Clipper 'Stad Amsterdam' (http://www.stadamsterdam.com) to re-enact the voyage of 'Darwin's' Beagle. The clipper, which is a modern replica of the Cutty Sark, left Plymouth (UK) on September 1, 2009 on an eight-month voyage that re-traced Darwin's original journey. Three of us (AAG, JK and WJB) were on board during some legs of the trip. A dust storm began on September 21, 2009 while the 'Stad Amsterdam' was anchored in Praia, the Capital of the Cape Verde Islands (14° 55′ 15″ N, 23° 30′ 30″ W). Collection began on the top of the main mast at 11:00 GMT with a surface-sterilised hand-held Dyson vacuum cleaner. Sampling was repeated at 19:45 GMT the next day (14° 07'13' N, 23° 43' 65" W) at the same position on the mast. Sterile aluminium foil was also laid out at the foot of the main mast as well as the roofs of the port- and starboard-side toilettes. Sterile 50 ml Falcon centrifuge tubes were opened and placed in eight separate locations around the deck of the 'Stad Amsterdam'. One day later the tubes were closed, the aluminium foil folded up and prepared for shipment to Switzerland for analysis. Since exposed aluminium foil on top of the port- and starboard-side toilets collected the most dust, this method and location was used again on September 23 when the 'Stad Amsterdam' was at sea (N11°33°W). Controls consisted of aluminium foil laid out at the same locations and exposed for the same time but on September 27, well after the dust storm had ended.

analyses (using XLSTAT2011 software) performed on the transformed correlation matrices (Addinsoft, New York, NY, USA).

Results

Geochemistry

Sand was analysed for both major and trace elements (see Figure 2a, b). Dust from the Bodélé Depression was only analysed for major elements. With the exception of C3 (64 weight (wt) %), all sand samples contained at least 85 wt % SiO_2 (in wt % respectively: C1 93; C2 85; C4 95; C5 89; C6 87; C7 87; C8 92 and C9 89). Mineral contents varied widely and without association with location (for example, Fe). Sample C4 contained 0.2 wt % Fe_2O_3, whereas C3 had more than 5.2 wt % (median around 3 wt %) (see Bristow *et al.*, 2010). Consequently, correlations between geographical location and Fe

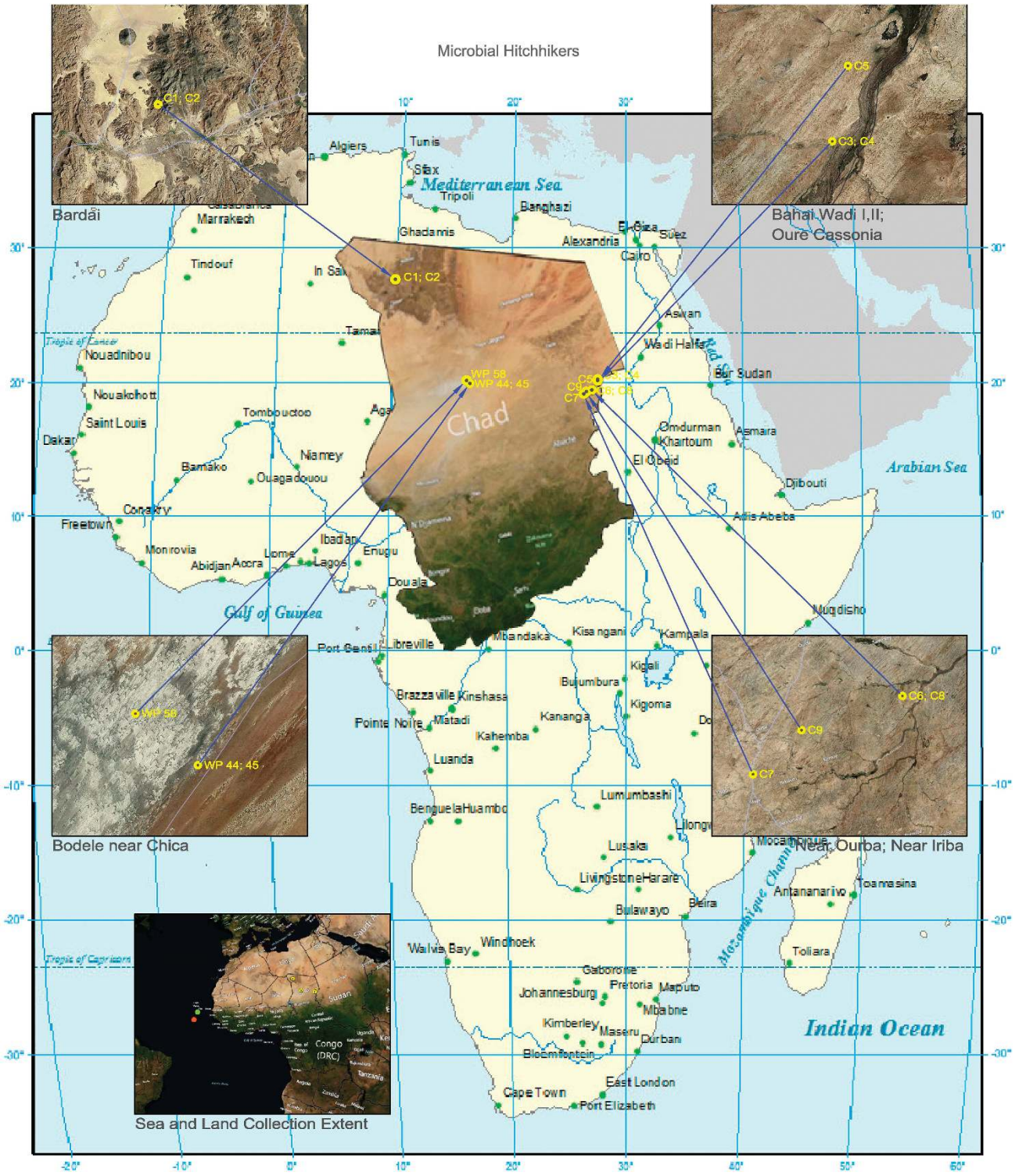


Figure 1 Map of Africa with insets showing the dust/sand collection sites in the Republic of Chad and the Cape Verde Islands. Abbreviations C1—C9 represent the sand collection sites in Chad (see Table 1). WP44, WP45 and WP58 show those in the Bodélé Depression (Table 2) whereas insert (e) shows the positions of the 'Stad Amsterdam' near the Cape Verde Islands during the storm in which dust was collected (Table 3).

content could not be drawn (vide infra). The scarcity of phosphorus was particularly striking: none was found in C4 and C7 whereas C1 contained only 0.002 wt %. C3 had significantly larger values (0.13 wt %) and Bodélé dust was enriched in P (between 0.06 and 0.09 wt %). Similarly, C3 had higher values of Al, Fe, K and P than the other samples. Zirconium was the most abundant metal: concentrations ranged

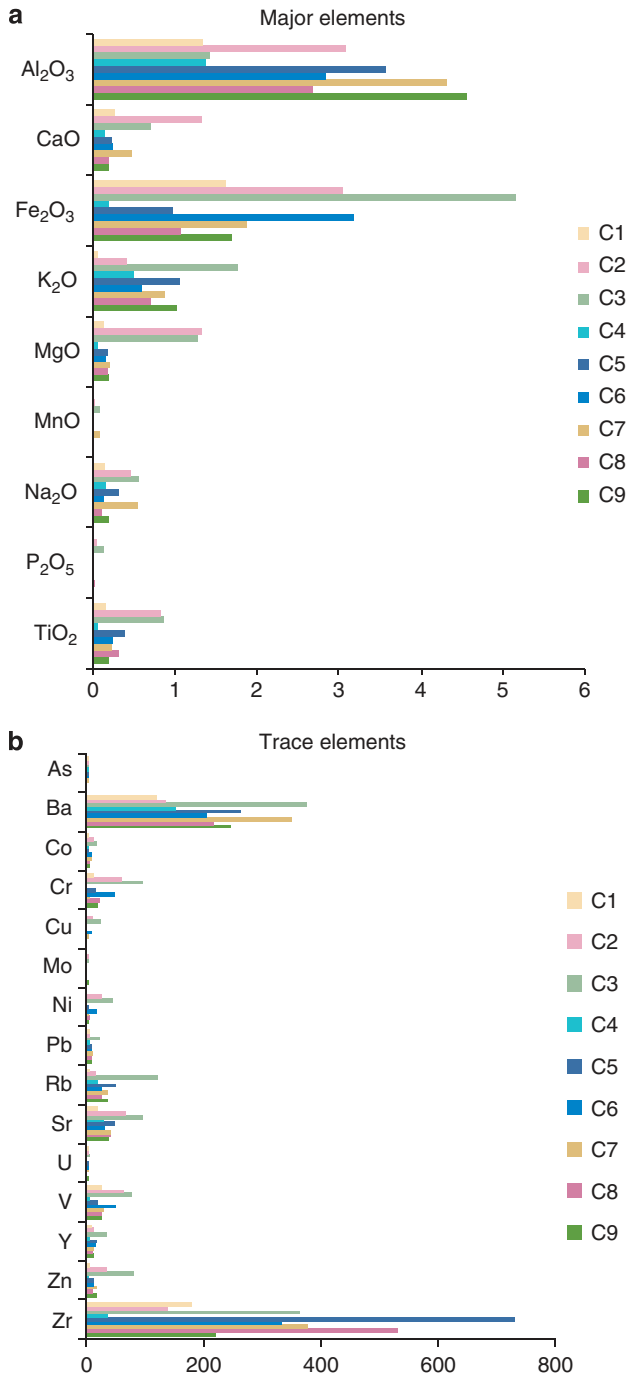


Figure 2 Elemental analyses of desert material collected from the Republic of Chad. (**a**) Major elements are listed in weight per cent, (**b**) Trace elements in mg kg^{-1}.

from ~40 mg kg^{-1} (C4) to 730 mg kg^{-1} (C5). Variation in the levels of barium, the next most abundant metal, was much less (from 120 mg kg^{-1} in C1 to 350 mg kg^{-1} in C7).

Increased elemental loads in dust can be explained by fractionation of soils and sediments during transport by wind. Strong winds are known to enrich dust with Zr, but it is also possible that the rocks from which the sand/dust originated, contained granites that are known to have high zircon contents.

Microscopic structure

SEM showed, with the exception of C3, that the non-rehydrated samples were a collection of loose grains to which small particles adhered (Figure 3). Under these *in situ* conditions, it was difficult to distinguish microbes from particles of clay, diatom fragments and other adhering material. Nevertheless, obvious cellular structures, often residing in surface irregularities that offer microbes dwelling spaces, were visible (Figure 3). C2 is representative of most samples and clearly shows rounded grains produced by wind erosion (Figure 3a). Enlargement (Figure 3b) revealed the surface irregularities and encrustation that is typical of all samples (cf Chappell *et al.*, 2008). Fragments of diatom shells can be seen in close-ups (for example, Figure 3c). As both Figure 3a and Figure 3d are reproduced at the same magnification, the difference between loose grains (in all other samples) and the surface crust in C3 is readily apparent. Other noteworthy features include: many, diverse particles that closely resemble fragments formed by wind in C4 (Figure 3e); a bacterial colony on the surface of a depression in the grain of C5 (Figure 3f); an encrusted microbial filament on the surface of the sand grain in Figure 3g (C6) and; a branching fungal hyphae in close contact with the sand grain surface as well as particulate matter held together by a slimy matrix (Figure 3h—C5).

Microbes in Chad sand

Two different HtS techniques were used: with all the samples except C3 and C5, DNA was extracted from the sand or dust, amplified using primers for 16 S rRNA genes and the products subjected to HtS. With samples C3 and C5, the isolated DNA was directly sequenced (see Giongo *et al.*, 2012). Except for C3 and C5, where classified reads (read = a single, unedited, machine-generated DNA sequence) were used, the results are given in per cent of total reads. The number of reads obtained varied widely: C1 gave only 351; C2 yielded 26 239; C4 89 811; C6 98 718; C7 50 945; C8 159 088 and C9 30 077 (classified reads for C3 and C5 were 5136 and 5319 respectively). Despite the >70-fold smaller numbers of reads obtained for C1 and C2, numerous genera that were present in these samples were absent in samples C3—C9.

Phylum actinobacteria

Forty-nine families were identified, some of which were present in most samples, but their numbers varied greatly. Supplementary Table 1 lists those families comprising ⩾0.3% of total reads and the seven most abundant families are shown in

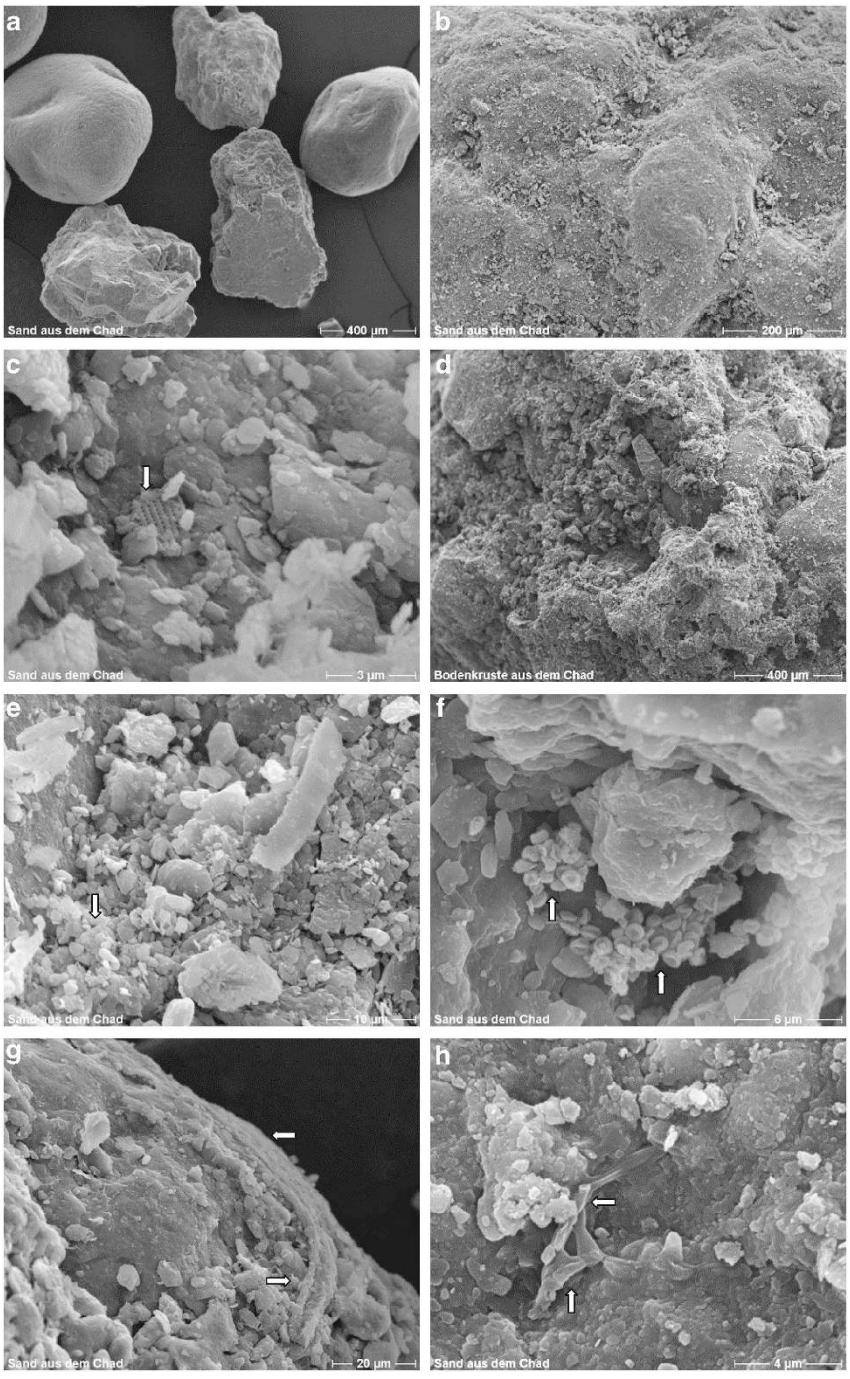


Figure 3 SEM micrographs of air-dried samples of surface material from Chad: Panel (**a**) = C2; (**b**) = C1; (**c**) = C1 magnified; (**d**) = C3; (**e**) = C4; (**f**) = C5; (**g**) = C6 and (**h**) = C5. Except for C3, where sand particles were held together by a thick layer of biological material, all other samples were collections of separate sand grains. A great variety of particulate matter can be seen attached to the grains: fragments of diatom shells (arrow) in (**c**); clumped microbial cells (arrow) in (**e**); collapsed microbial cells (possibly Archea – arrows in (**f**); mineral-encrusted microbial filaments (possibly fungal hyphae) in (**g**) and branched fungal hyphae (arrows) in (**h**). Different ratios of particulate to biogenic matter and sand grains can be seen in D, where the grains in sample C3 seem to be 'cemented together' into a surface crust (cf samples C2 (**a**) and (**b**) (C1)).

Figure 4a. Many members of these families are thermophilic, others produce antibiotics and all are widespread in soils. Due to the high abundance of digestive tract bacteria (Bifidobacteriaceae), C1 was strikingly different from the other samples, whereas the other six families were abundant in all samples.

Phylum Firmicutes

Twenty-eight families of Firmicutes were identified but their presence in individual samples varied greatly (Supplementary Table 2 lists families that make up $\geqslant 0.5\%$ of the total reads). The seven most abundant families are presented in Figure 4b.

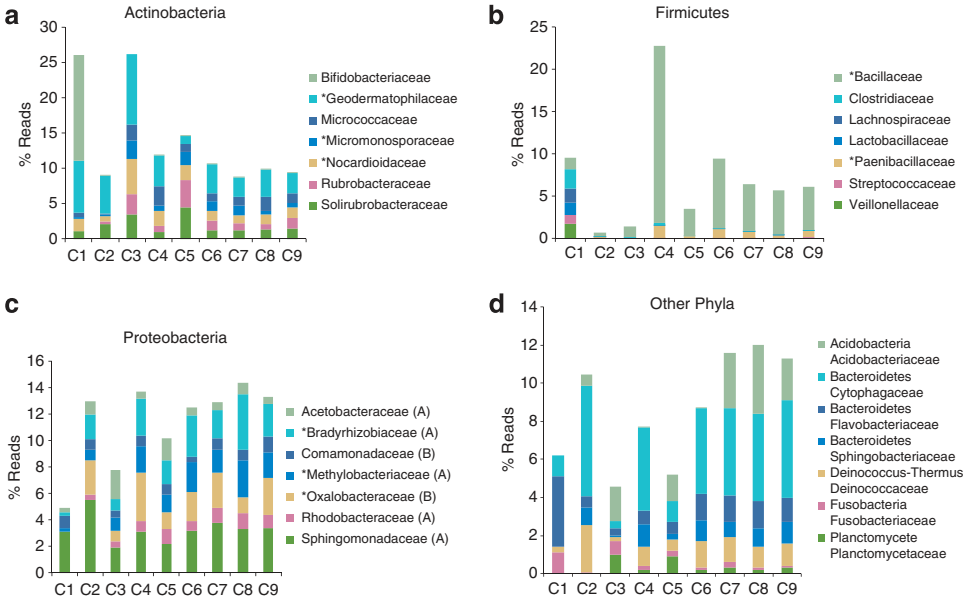


Figure 4 (**a**) The seven most abundant families of the Phylum Actinobacteria found in the sands of Chad as revealed by HtS (Actinobacterial families making up $\geqslant 0.3\%$ of the total 'reads' are listed in Supplementary Table 1). Families marked with an asterisk were also found by culture-based methods. Note that the composition of C1 is markedly different from the other samples—more than 50% of the Actinobacteria are Bifidobacteriaceae (anaerobic bacteria of the gastrointestinal tract and vagina), that along with the many Enterobacteriaceae in this sample, undoubtedly came from the large military force garrisoned nearby. (**b**) HtS based-data showing the seven most dominant families of the Phylum Firmicutes. Families marked with an asterisk were also found by culture-based methods (Supplementary Table 2 lists those families that comprise $\geqslant 0.5\%$ of the total 'reads'). Again it is obvious that the bacterial composition of C1 differs markedly from the others. Significant numbers of Clostridiaceae (anaerobic to oxygen-tolerant spore-forming bacilli found in soil as well as in normal intestinal flora of animals), Lachnospiraceae (colon inhabitants), Lactobacillaceae (gastrointestinal tract), Streptococcaceae (widely distributed) and Veillonellaceae (gastrointestinal tract) were found. Undoubtedly, their presence is due to human activity nearby. (**c**) HtS based-data showing the seven most dominant families of the Phylum Proteobacteria. Families marked with an asterisk were also found by culture-based methods (Supplementary Table 3 lists those families that comprise $\geqslant 0.3\%$ of the total 'reads'). Note that two families that were fairly abundant in samples C2 to C9 were not found in C1. The most prominent of these were the Oxalobacteraceae (that includes the soil/rhizosphere inhabitants *Duganella*, *Herbaspirillum*, *Naxibacter*, *Oxalicibacterium* and *Telluria*). *Herbaspirillum seropedicae* is a well-studied nitrogen-fixing bacterium. Rhodocacteraceae (non-sulphur, purple, photoheterotrophic bacteria) were also noticeably absent. (**d**) HtS based-data showing the other seven predominant phyla/families apart from those listed in Figure 4 A–C that make up $\geqslant 0.3\%$ of the total 'reads' in Chad sand (further details are given in Supplementary Table 4). Here again, the bacterial composition of C1 was noticeably different from the rest. Acidobacteria, a widespread and diverse group of mostly soil inhabitants; the Sphingobacteriaceae comprising three genera of environmental bacteria (*Mucilaginibacter*, *Pedobacter* and *Sphingobacterium*) and the Planctomycetaceae, soil and water (both fresh and hyper-saline) bacteria were not detected.

Bacillaceae dominate in eight of the nine samples comprising $>5\%$ of the reads in C4, C6, C7, C8 and C9. Despite the small number of reads obtained, C1 was notably different with 0.1% Enterococcaceae and $\geqslant 1\%$ Clostridiaceae, Lachnospiraceae, Lactobacillaceae and Veillonellaceae.

Phylum Proteobacteria

Comprising representatives of 71 families, the Proteobacteria was the most important phylum of bacteria found (Supplementary Table 3, Figure 4c). All divisions were represented: α-Proteobacteria (22 families); β-Proteobacteria (11 families); γ-Proteobacteria (18 families); δ-Proteobacteria (17 families); ε-Proteobacteria (2 families), Campylobacteraceae (traces in C8 and C9), Helicobacteraceae (traces in C8) and ζ-Proteobacteria (traces of Mariprofundaceae in C6).

Some families were abundant in almost every sample: Bradyrhizobiaceae, Methylobacteriaceae and Sphingomonadaceae (α-Proteobacteria) and Oxalobacteraceae (β-Proteobacteria) for example. Other families were less abundant but well distributed. Less numerous families ($<0.3\%$) included: Bacteriovoraceae (δ); Bdellovibrionaceae (δ); Coxiellaceae (γ); Erythrobacteriaceae (α); Legionellaceae (γ); Methylocystaceae (α); Methylophilaceae (β); Neisseriaceae (β); Nitrosomonadaceae (β); Pasteurellaceae (γ); Phaselicystidaceae (δ); Phyllobacteriaceae (α); Polyangiaceae (α); Rhodobiaceae (α); Rhodocyclaceae (β); Rickettsiaceae (α); Sinobacteriaceae (γ) and Xanthobacteraceae (α).

Other phyla

Low numbers of 18 other phyla were also found (Supplementary Table 4, Figure 4d)—two Archaea: Crenarchaeota (Desulfurococcaceae in C7; Sulfolobaceae in C4 and Thermofilaceae in C5) and Euryarchaeota (Methanothermaceae in C5). Acidobacteria comprising three families, Bacteroidetes

(three dominant families), Deinococcus—Thermus (2), Fusobacteria (1) and Planctomycetes (1).

Fungi

Ascomycota were overwhelmingly dominant followed by Basidiomycota with traces of Chytridiomycota, Microsporidia and Glomeromycota (Figure 5).

Ascribing 'reads' to genera

About 1 in 10 000 reads could be ascribed to a genus in 13 phyla yielding 453 genera (Table 4). Actinobacteria were both well distributed and abundant. *Blastococcus, Geodermatophilus* (both Geodermatophilaceae); *Arthrobacter* (Micrococcaceae); *Nocardioides* (Nocardioidaceae); *Rubrobacter* (Rubrobacteraceae) and *Solirubrobacter* (Solirubrobacteraceae) were the principal genera found. Other well-distributed genera included: *Actinoplanes, Cellulomonas, Conexibacter, Modestobacter, Iamia, Kineococcus, Kineosporia, Kocuria, Marmoricola, Micromonospora, Pseudonocardia* and *Streptomyces.*

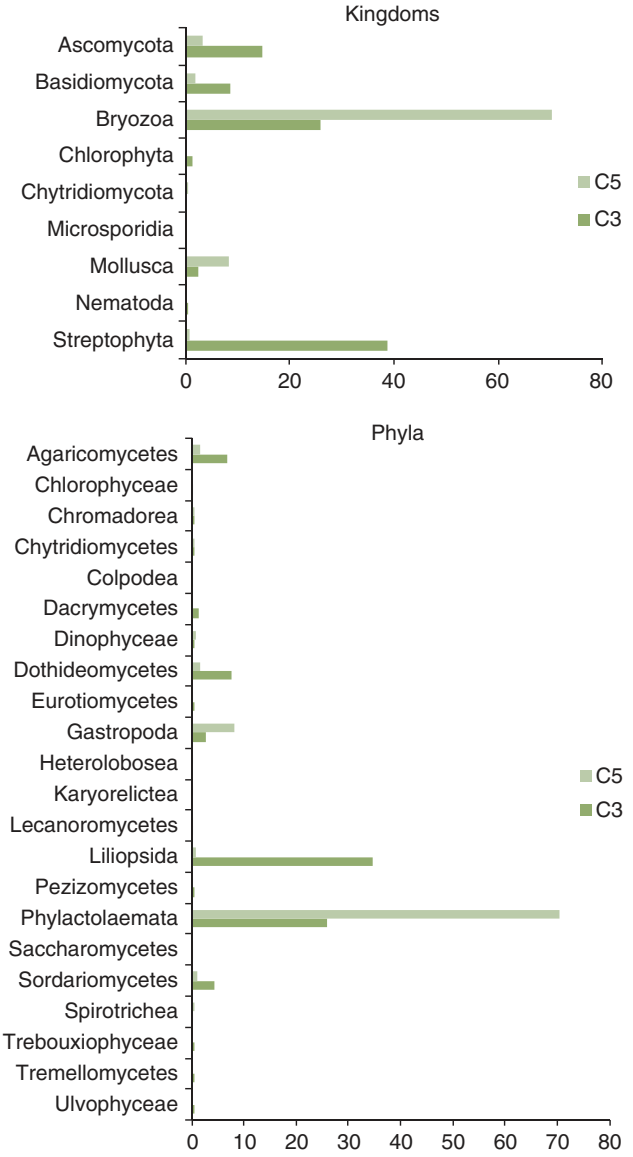


Figure 5 Kingdoms and phyla of Eukaryotes found in samples C3 and C5 by direct HtS methods.

Bacillus was the principal genus of the Firmicutes (% reads in C1 to C9: 1.1; 0.4; 1.2; 16.9; 2.7; 6.6; 4.9; 4.3 and 4.3 respectively). Other important genera included *Ammoniphilus* and *Paenibacillus.*

Very diverse proteobacterial populations (172 genera in total) were present, but most comprised <1% of reads. Nevertheless, three abundant and well-distributed genera were found: *Balneimonas* (now called *Microvirga*), *Methylobacterium* (both Methylobacteriaceae) and *Sphingomonas* (Sphingomonadaceae). Less abundant but well distributed were: *Acidovorax, Afipia, Azospirillum* (Beijerinckiaceae), *Belnapia, Bosea, Bradyrhizobium, Burkholderia, Chelatococcus, Cystobacter, Duganella, Herbaspirillum, Janthinobacterium, Kaistobacter, Massilia* (Oxalobacteraceae), *Rhodomicrobium, Rubellimicrobium, Sinorhizobium, Sphingosinicella* and *Variovorax* (various α- and β-Proteobacteria).

Other phyla that contained named genera included Bacteroidetes with Bacteroidaceae (*Bacteroides*) and Cytophagaceae (*Adhaeribacter, Flexibacter*, *Hymenobacter* and *Sporocytophaga*). Less abundant genera in the Flavobacteriaceae and Sphingobacteriaceae as well as *Deinococcus* (Deinococcus-Thermus) and *Nitrospira* (Nitrospirae) were also present. Other well-distributed genera included *Acidimicrobia* (Cyclobacteriaceae), *Hymenobacter* and *Sporocytophyga* (Flavobacteriaceae).

The Proteobacterial family Enterobacteriaceae contains a number of animal pathogens, some of which were occasionally found in most samples (< 0.1% of reads): C1 had more *Enterobacter, Escherichia* and *Shigella* (but still less than 1%) than the other samples. *Klebsiella, Salmonella* and *Serratia* were also present. Traces of the phytopathogen *Erwinia* were found in C1 as well as in C7–C9.

HtS techniques applied to DNA isolated from samples C3 and C5 allowed sequenced-based insights into the eukaryotes present (Figure 5). Amongst the fungi, Ascomycota were almost twice as abundant as Basidiomycota but traces of Chytridiomycota, Microsporidia and Glomeromycetes were also present. Bryozoa (aquatic, invertebrate animals) and Streptophyta (land plants and green algae) were also prevalent. Mollusca, which includes terrestrial representatives, was the other important group and a few green algae (see below) were also present.

Living microorganisms

Bacteria. In accordance with HtS data, samples C1 and C2 were poorer in bacteria than the others

(Table 5). Both were collected at Bardaï, northern Chad (Figure 1, Table 1). Spores usually comprised <30% of total colony-forming units (CFU), but bacteria that form an endospore were the dominant cultivable prokaryotes (see Table 5). Conversely, there were proportionally fewer Bacillaceae in Bodélé dust. It should not be forgotten however that Bacillaceae grow faster than other bacteria and it is thus difficult to isolate other genera in their presence. Thus the 'total numbers' presented here are estimates based on individual colonies that permits comparisons between comparable samples (for example, the sand samples, but not the dust). Nevertheless, half as many *Bacillus* spp. were present in the sands of Chad than were found aboard the 'Stad Amsterdam' (Table 6). Usually different species were present in the two locations, but *B. pumilus* and *B. subtilis* were found at both. The living bacterial population of Bodélé dust was different from that found in the other locations. Only *B. subtilis* and *Kocuria* spp. (both environmental bacteria) were omnipresent. HtS data show that many environmental bacteria (anaerobic, autotrophic, photosynthetic and thermophilic) that cannot be isolated on standard media were present in the samples (Supplementary Tables 1 to 4), but unfortunately, the presence of intact DNA is not proof that the organism that furnished it is still alive.

Acacia spp., trees of the legume subfamily Mimosoideae, are a feature of African (and Australian) deserts. To see if bacteria that provoke the formation of root nodules on legumes were present in the sands of Chad, we used selected African species of *Acacia* as 'traps' to isolate rhizobia (see Giongo *et al.*, 2012). *Bradyrhizobium* spp., normal symbionts of Acacias (Dreyfus and Dommergues, 1981) were isolated this way. Diazotrophic *Azospirillum* spp. (Rhodospirillaceae) were also isolated. Use of a medium for the isolation of nitrifying bacteria permitted the cultivation of *Nitrospira* spp. HtS-based techniques confirmed the presence of all cultivable families but did not provide evidence for *Agrobacterium*, *Exiguobacterium* and *Tsukamurella* (cf Table 6, Supplementary Tables 1 to 4).

Living fungi

Although HtS-based methods showed that about half the fungi present in samples C3 and C5 were Basidiomycota, none were isolated in culture (Table 7). Common environmental genera like *Aspergillus* and *Trichoderma* were present along with the endophyte *Fusarium equiseti* (Macia-Vicente *et al.*, 2009). Only *Cochliobolus lunatus* was widespread. Whether or not a particular fungal species was present in a sample varied much more than with bacteria: most fungi were found in samples C3, C8 and C9. Interestingly, the only fungus found in Chad and on the 'Stad Amsterdam',

Table 4 Number of bacterial families and genera (grouped by Phylum) that make up ≥0.01% of total 'reads'' in the nine samples of Chad sand

Phylum	*Number of: Families*	*Genera*	*Number of reads: 0.1–0.9%*	*≥1%*	*≥1% in at least 3 samples*
Actinobacteria	44	150	39	13	6
Acidobacteria	3	17	8	0	0
Bacteroidetes	8	29	13	5	2
Chloroflexi	6	9	2	0	0
Deinococcus-Thermus	2	2	0	1	1
Firmicutes	15	44	25	4	1
Fusobacteria	1	4	1	1	0
Gemmatimonadetes	1	1	1	0	0
Nitrospirae	1	2	1	0	0
Proteobacteria	44	172	64	6	3
Planctomycetes	1	10	1	0	0
Tenericutes	2	2	1	0	0
Verrucomicrobia	2	11	2	0	0

Table 5 Numbers of viable bacteria in Chad sand samples estimated by culture-dependent methods

	# Bacteria (CFU g^{-1})								
	C1	*C2*	*C3*	*C4*	*C5*	*C6*	*C7*	*C8*	*C9*
Total # OTU	9×10^2	5.4×10^3	2.2×10^6	7.4×10^4	2.2×10^4	1.5×10^5	1.2×10^5	3.6×10^5	5.4×10^5
# Total spores	2.8×10^1	2.8×10^1	2.2×10^5	2×10^4	6.2×10^3	1.2×10^3	7×10^2	1.9×10^3	5.6×10^2
% spores	3.1	0.5	10.0	27.0	28.2	0.8	0.6	0.5	0.1
# OTU identified	5	7	13	8	10	10	16	5	6
Location	Bardaï	Bahaï	Bardaï Wadi I	Bahaï Wadi II	Oure Cassoni	Ourba	Iriba	Ourba	Ourba

Abbreviation: OTU, operational taxonomic unit.

Table 6 Living bacteria isolated by culture-dependent methods from sand and dust in Africa

Bacteria	*Chad sand*	*Bodélé dust*	*'Beagle' dust*
[a]*Agrobacterium tumefasciens*	C4		
Arthrobacter dextranolyticus	C7		
A. globiformis	C2		
Arthrobacter spp.	C2, C7, C9	WP44	
[a]*Azospirillum* spp.	C7		
Bacillus badius			F3,A
B. casamensis	C2		
B. circulans	C6		
B. drentensis	C3		
B. endophiticus	C4		F3, F4
B. firmus			F7
B. foraminis	C1, C2		
B. funiculus	C6		
B. horikoshii			F4
B. massiliensis	C7		
B. megaterium	C4, C5, C6, C7, C8		
B. nearlsonii			F1, A, B, M
B. niabensis			F4
B. niacini			D
B. pocheonensis	C7		
B. pumilus	C3		F2,F4
B. subtilis	C2, C3, C4, C5, C6, C7, C8, C9	WP44	F1, F2, F4, A, B, M
B. thuringiensis	C3		
Bacillus spp.	C2, C3, C5, C6, C7, C8		
Blastococcus spp.		WP44, WP58	
[a]*Bradyrhizobium* spp.	C4, C5, C7	WP58	
Brevibacillus borstelensis	C1		
Chelatococcus asacharovorans	C9		
[a]*Cohnella* spp.	C5		
Cutobacterium citreum	C3		
Curtobacterium spp.	C6, C7		
Duganella violaceusniger	C4, C5, C7		
Exiguobacterium spp.	C5, C9		
Geodermatophilus obscurus	C5		
Geodermatophilus spp.	C9		
Hymenobacter spp.	C7		
Kocuria spp.	C1	WP45, WP58	F4
Massilia timonae		WP45	
Micromonospora spp.			D
Microvirga spp.		WP58	
Neisseria spp.			D
Nitrospira multiformis	C7, C8		
Nitrospira spp.	C4, C6		
Nocardioides kribbensis		WP45	
Oceanobacillus picturae	C4		
Ornithimicrobium humiphilum		WP58	
Oxalobacteraceae	C7		F4
Oxalobacter spp.	C4		
Paenibacillus macerans			A
[a]*P. phyllosphaerae*	C4		
P. validus			F7
Paenibacillus spp.	C8		
Planococcus spp.	C6		
Pontibacter spp.	C9		
Pseudomonas luteola	C5		
[a]*Rhizobium* spp.	C7		
Rothia spp.	C1		
Shigella/Escherichia		WP44	
Sinorhizobium spp.	C5		
Sphingomonas yabuuchaie	C3		
Sporosarcina spp.	C6		
Streptomyces diastaticus	C3		
S.djakartensis	C2		
S. lilaceus	C7		
S. pseudogriseolus	C5		
S. purpurescens	C5		
S. rochei	C3		
S. violaceoruber	C3		
Terribacillus halophilus			F3
T. shanxiensis	C8		
Tsukamurella spp.		WP45, WP58	
Virgibacillus picturae	C4		

For abbreviations see Tables 1 to 3.
[a]'Trapped' using leguminous plants as 'bait'. 'Beagle' dust means dust collected on board the 'Stad Amsterdam' that re-enacted the voyage of Charles Darwin's Beagle in 2009 (see Figure 1 and Table 3).

Cochliobolus lunatus, is possibly a pathogen of sugarcane.

Living algae

Algal isolates were obtained from samples C2, C3, C5 and C6. Morphological observations suggested that the isolates from C2 and C3 belonged to the *Chlorella* morphotype [cf Ettl, Gärtner (1995); John *et al.,* 2002; Luo *et al.*, 2010], whereas the isolates from C5 and C6 were similar to *Haematococcus pluvinalis* Flotow. Sequenced parts of the 3'-18 S rRNA gene and the entire internal transcribed spacer region supported the morphological observations. Isolate C2 was identical to that of C3 (both 97% identity with *Micractinium reisseri*—Chlorellaceae, Trebouxiophyceae), the 'European' algal symbiont of *Paramecium bursaria* (Hoshina *et al.*, 2010). The internal transcribed spacer sequences of C5 and C6 were 98% similar to each other. The closest match in Genbank was GQ463618.1 that represents *Haematococcus pluvialis* strain H but they are not conspecific. Further work is required to assign the clones from Chad to a species of *Haematococcus*, as the phylogeny of this cosmopolitan group is unclear. The six species—*H. buetschlii* Blochmann, *H. capensis* Pocock, *H. carocellus* R.H.Thompson and D.E.Wujek, *H. droebakensis* Wollenweber, *H. pluvialis* Flotow and *H. zimbabwiensis* Pockock, are all freshwater inhabitants.

Table 7 Living fungi isolated by culture-dependent methods from sand and dust in Africa

Species from Chad sand	*Sample*	*Classification (Family, Order, Phylum)*
Ampelomyces sp.	C9	Leptosphaeriaceae; Pleosporales, Ascomycota
Aspergillus flavus	C3	mitosporic Trichocomaceae, Eurotiales, Ascomycota
Aspergillus niger	C8	mitosporic Trichocomaceae, Eurotiales, Ascomycota
Capnobotryella sp.	C2	Teratosphaeriaceae, Capnodiales, Ascomycota
Cochliobolus lunatus	C3, C4, C7, C8, C9	Pleosporaceae, Pleosporales, Ascomycota
Eurotium chevalieri/amstelodamii	C8	Trichocomaceae, Eurotiales, Ascomycota
Exserohilum rostratum	C9	Pleosporaceae, Pleosporales, Ascomycota
Fusarium equiseti	C3	Mitosporic Hypocreales, Ascomycota
Fusarium sp.	C3, C9	Mitosporic Hypocreales, Ascomycota
Humicola fuscoatra	C9	Mitosporic Ascomycota
Myrothecium verrucaria	C8	Mitosporic Hypocreales, Ascomycota
Phoma macrostoma	C8	Mitosporic Ascomycota
Pleiochaeta ghindensis	C9	Mitosporic Pezizomycotina, Ascomycota
Teratosphaeria sp.	C2	Teratosphaeriaceae, Capnodiales, Ascomycota
Trichoderma inhamatum	C3	Hypocreaceae, Hypocreales, Ascomycota
Ulocladium sp.	C5	Pleosporaceae, Pleosporales, Ascomycota
Westerdykella nigra	C7	Sporomiaceae, Pleosporales, Ascomycota
Species from 'Beagle' dust	*Sample*	*Taxonomic position*
Alternaria alternata	F2	Pleosporales, Pleosporaceae, Ascomycota
Bipolaris spicifera	F2	Pleosporales, Pleosporaceae, Ascomycota
Bipolaris spicifera or *Cochliobolus australiensis*	F3, F4	Pleosporaceae, Pleosporales, Ascomycota
Cladosporium sphaerospermum or *Cladosporium lignicola*	F4, F4	Davidiellaceae, Capnodiales, Ascomycota
Cochliobolus lunatus or *Macrophomina phaseolina*	F1, F2, F4, F4	Pleosporaceae, Pleosporales, Ascomycota or Botryosphaeriaceae, Botryosphaeriales, Ascomycota
Emericella nidulans or *Emericella quadrilineata*	F4	Trichocomaceae, Eurotiales, Ascomycota
Phoma betae or *Macrophoma* sp.	F2	Mitosporic Ascomycota
Phoma sp.	F2	Mitosporic Ascomycota
Thielavia arenaria or *Thielavia subthermophila*	F2, A	Chaetomiaceae, Sordariales, Ascomycota

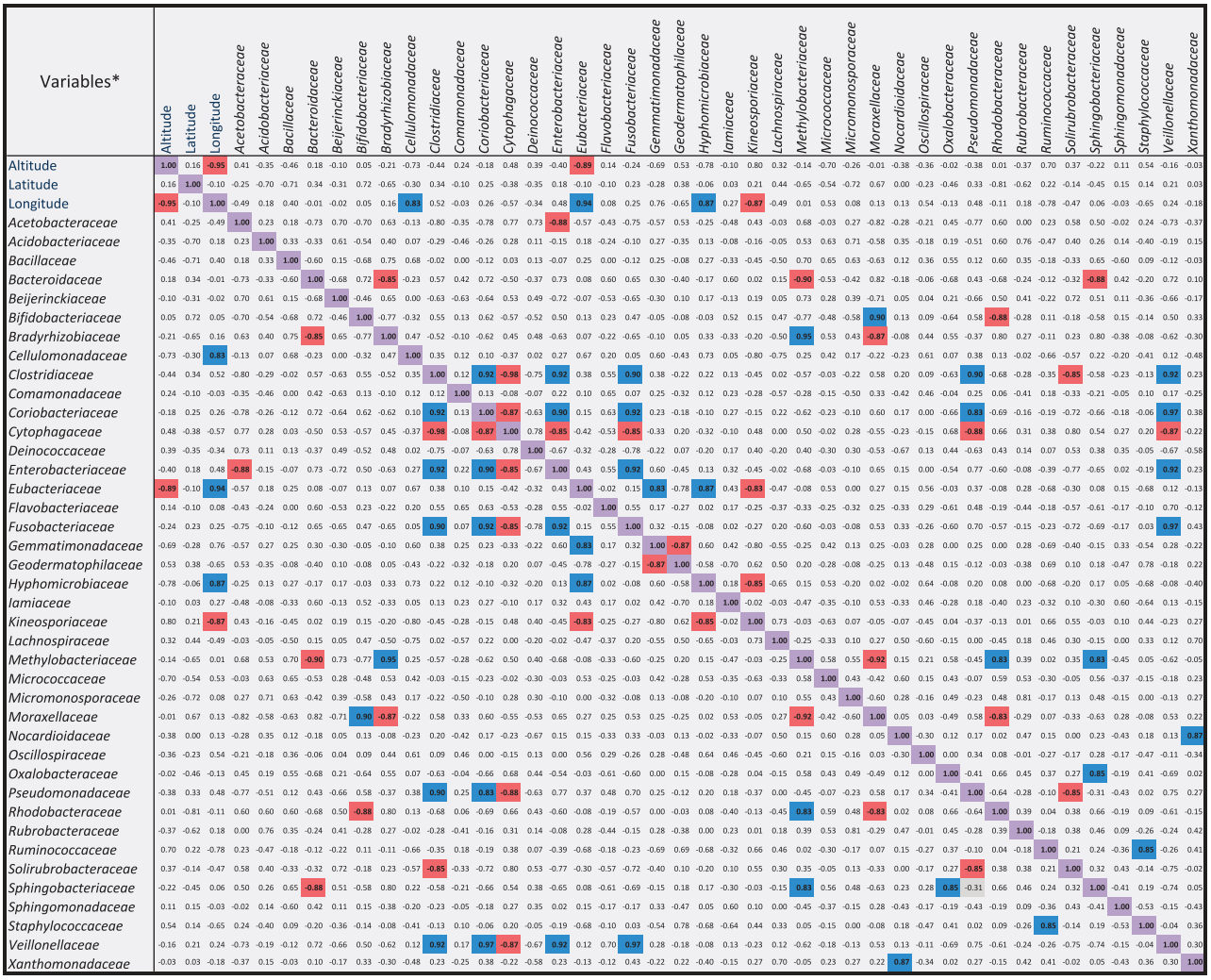
'Beagle' dust means dust collected on board the 'Stad Amsterdam' that re-enacted the voyage of Charles Darwin's Beagle in 2009 (Table 3).

HtS identified two phyla of Viridiplantae: Chlorophyta (1.3%) and Charophyta (0.2%). Two groups of Heterokontophyta—Bacilliarophyceae (diatoms) (0.04%) as well as the family Chrysophyceae (0.04%) were also present (Figure 5).

Statistical correlations and dependencies. We assumed that specific microbes prefer certain environments. To test this, we calculated Spearman correlations between the concurrence of selected families and geographical parameters including altitude, latitude and longitude. The Cellulomonadaceae, Eubacteriaceae and Hyphomicrobiaceae were concentrated in eastern Chad whereas the Kineosporiaceae were mostly in the west (Figure 6). The Eubacteriaceae frequented lowlands. Cytophagaceae, the fourth most abundant family, correlated negatively with Clostridiaceae and Enterobacteriaceae that were positively associated. The fifth most abundant family Bacteroidaceae, correlated negatively with Bradyrhizobiaceae, Methylobacteriaceae and Sphingobacteriaceae.

Few correlations could be drawn between the mineral content of the sand and the bacterial inhabitants, but occurrence of the second most cosmopolitan family, the Sphingomonadaceae, was highly correlated with presence of MgO, Sr, TiO_2 and Zn (Figure 7). On the other hand, the sixth most abundant family, Geodermatophilaceae, was negatively correlated with the presence of Ba, K_2O, Pb and Rb. This contrasts with the Gemmatimonodaceae that seemed to have a preference for Ba, MgO and Rb.

Principal component analyses reduced the variables (operational taxonomic units (OTUs) and geographic location) to two principal components or axes F1 and F2 (Figure 8). Component F1 had the largest negative loadings from Acetobacteraceae, Cytophagaceae, Methylobacteriaceae, Rhodobacteraceae and so on, which are highly specialised environmental bacteria. Large positive loadings of component F1 came from Clostridiaceae, Coriobacteriaceae, Enterobacteriaceae, Veillonellaceae and so on, and animal-associated bacteria in eastern Chad. Component F2 had the largest negative loadings from Cellulomonadaceae, Eubacteriaceae, Gemmatimonadaceae, Hyphomicrobiaceae and so on, which were common in eastern Chad. The Kineosporiaceae, Lachnospiraceae and Ruminococcaceae caused large positive loadings on F2 that were associated with northwestern Chad and higher elevations. Some families that clustered strongly (long arrows) were abundant at C1, C2 and C5 but rare at other sites. *Bacteroidaceae, Bifidobacteriaceae, Lachnospiraceae* and *Moraxellaceae* were



Values in bold are different from 0 with a significance level alpha=0.01

* Variables were stadardized (log10)

Figure 6 Spearman correlation matrixes between the 40 most abundant bacterial families detected by HtS methods as the principal variable with geographic coordinates and altitude as the supplementary variables. All variables were transformed using $\log_{10}$ to approximate a normal distribution. Red shading represents significant negative correlations; shading in blue signifies positive correlations ($\alpha < 0.01$). Note that the co-occurrence of some bacterial families is strongly positively or negatively correlated at the same location.

concentrated in central Chad. The *Cellulomonadaceae, Eubacteriaceae, Gemmatimonadaceae* clustered in eastern Chad. No particular family clustered at C3, C4, C6 and C7–9 (small arrows), indicating more random distributions.

Discussion

The Sahara, like other hot deserts of the intertropical zone, experiences temperatures up to 78 °C, as many as 3250 sunshine hours per year and <25 mm annual rainfall (Mainguet, 1995). Single daily variations in temperature of almost 40 °C (from −0.5 to +37.5 °C) have been recorded. Furthermore, the strong winds that are a feature of the Sahara mean that evaporation is greater than it would otherwise be. Merely to survive these extremes, desert dwelling microbes must possess a multitude of adaptations. Undoubtedly, this explains why up to 30% of living cells were in the form of spores (Table 5). We assume that most of the other living microbes were in a state of suspended animation that allows them to survive for extended periods (see Toepfer *et al.*, (2012)).

Application of an array of techniques revealed predominantly environmental micro-organisms that were well adapted to the desert. In a similar study, Chanal *et al.* (2006) analysed 117 clones from the Tatouine desert in Southern Tunisia and found that the distribution of the main phyla (Actinobacteria and Proteobacteria) was similar to that reported here (Figure 4a–c). Many Actinomycetes (Actinobacteria)

Variables	Al_2O_3	As	Ba	CaO	Co	Cr	Cu	Fe_2O_3	K_2O	MgO	MnO	Mo	Na_2O	Ni	P_2O_5	Pb	Rb	SiO_2	Sr	TiO_2	U	V	Y	Zn	Zr
Acetobacteraceae	-0.28	-0.20	-0.40	0.10	0.32	0.20	0.24	0.18	-0.52	0.18	0.27	-0.04	-0.32	0.27	0.06	-0.24	-0.51	-0.13	-0.02	0.03	-0.66	0.26	-0.30	0.08	-0.25
Acidobacteriaceae	0.44	-0.38	0.60	0.08	0.57	0.39	0.44	0.49	0.48	0.35	0.59	0.26	0.13	0.51	0.31	0.69	0.48	-0.45	0.34	0.31	0.03	0.49	0.30	0.44	0.33
Bacillaceae	-0.17	-0.33	0.15	-0.64	-0.31	-0.47	-0.26	-0.28	0.07	-0.52	-0.15	-0.57	-0.37	-0.33	-0.60	-0.01	0.10	0.35	-0.44	-0.50	-0.47	-0.37	-0.20	-0.40	0.05
Bacteroidaceae	0.37	0.40	0.33	0.44	0.03	0.02	0.04	0.13	0.37	0.24	0.13	0.13	0.48	-0.08	0.09	0.33	0.39	-0.23	0.27	0.27	0.74	0.10	0.50	0.27	0.40
Beijerinckiaceae	0.04	-0.20	-0.03	0.02	0.57	0.68	0.40	0.42	-0.03	0.26	0.33	0.29	-0.31	0.73	0.55	0.12	-0.05	-0.32	0.25	0.39	-0.26	0.47	0.07	0.27	0.00
Bifidobacteriaceae	0.05	0.54	-0.13	0.20	-0.11	0.20	-0.07	0.03	0.07	-0.05	-0.30	0.29	0.13	0.02	0.28	-0.15	0.04	0.03	0.03	0.15	0.63	-0.01	0.20	0.00	-0.07
Bradyrhizobiaceae	-0.20	-0.52	-0.05	-0.59	-0.08	-0.05	-0.20	-0.18	-0.07	-0.25	-0.07	-0.35	-0.58	0.07	-0.13	-0.05	-0.08	0.23	-0.23	-0.22	-0.61	-0.18	-0.28	-0.27	0.05
Cellulomonadaceae	-0.05	-0.14	0.32	-0.54	-0.34	-0.27	-0.24	-0.43	0.35	-0.39	-0.27	-0.51	-0.18	-0.14	-0.29	0.11	0.38	0.28	0.01	0.00	-0.18	-0.48	0.20	-0.35	0.42
Clostridiaceae	-0.17	0.52	0.13	-0.08	-0.40	-0.28	-0.20	-0.27	0.18	-0.49	-0.42	-0.35	0.03	-0.37	-0.28	-0.03	0.22	0.32	-0.16	-0.07	0.26	-0.38	0.22	-0.37	0.25
Comamonadaceae	0.05	-0.36	0.13	-0.38	-0.61	-0.60	-0.73	-0.60	0.18	-0.13	-0.30	-0.41	-0.03	-0.60	-0.36	0.07	0.15	0.40	-0.18	-0.35	0.11	-0.59	-0.17	-0.30	0.35
Coriobacteriaceae	-0.05	0.64	0.15	0.13	-0.22	-0.20	-0.07	0.00	0.17	-0.37	-0.20	-0.28	0.08	-0.31	-0.29	0.04	0.22	0.13	-0.21	-0.08	0.42	-0.14	0.32	-0.18	0.25
Cytophagaceae	0.23	-0.48	-0.08	0.15	0.43	0.22	0.26	0.32	-0.17	0.53	0.50	0.35	0.08	0.32	0.19	0.07	-0.18	-0.38	0.16	0.03	-0.21	0.42	-0.17	0.43	-0.27
Deinococcaceae	0.22	-0.12	-0.03	0.23	0.47	0.28	0.37	0.37	-0.10	0.44	0.53	0.10	0.03	0.40	0.10	0.07	-0.07	-0.47	0.23	0.23	-0.13	0.42	0.20	0.43	0.00
Enterobacteriaceae	0.18	0.39	0.43	0.09	-0.23	-0.27	-0.05	-0.10	0.43	-0.19	-0.12	-0.17	0.37	-0.30	-0.24	0.27	0.48	0.05	0.05	0.03	0.50	-0.19	0.42	-0.07	0.37
Eubacteriaceae	0.43	-0.29	0.62	-0.43	-0.16	0.05	-0.13	-0.25	0.77	-0.03	-0.17	0.13	0.22	0.13	0.22	0.43	0.74	0.03	0.35	0.25	0.40	-0.25	0.38	0.00	0.38
Flavobacteriaceae	-0.13	0.17	0.18	-0.12	-0.39	-0.38	-0.47	-0.32	0.17	-0.31	-0.20	-0.64	-0.22	-0.45	-0.36	0.17	0.18	0.32	-0.21	-0.13	0.11	-0.40	0.13	-0.33	0.67
Fusobacteriaceae	-0.07	0.47	0.22	0.16	-0.23	-0.28	-0.05	-0.07	0.17	-0.30	-0.17	-0.24	0.20	-0.37	-0.29	0.12	0.21	0.15	-0.13	-0.08	0.29	-0.17	0.20	-0.18	0.23
Gemmatimonadaceae	0.62	-0.31	**0.85**	-0.14	-0.04	-0.13	0.02	-0.13	**0.85**	0.19	0.17	0.06	0.55	0.01	0.01	0.67	**0.87**	-0.18	0.48	0.27	0.45	-0.13	0.53	0.22	0.55
Geodermatophilaceae	-0.78	0.33	**-0.92**	0.10	-0.15	-0.17	-0.04	-0.07	**-0.98**	-0.35	-0.30	-0.20	-0.43	-0.27	-0.29	**-0.83**	**-0.97**	0.33	-0.56	-0.43	-0.66	-0.07	-0.68	-0.38	-0.73
Hyphomicrobiaceae	0.12	-0.40	0.37	-0.58	-0.28	0.07	-0.31	-0.47	0.53	-0.14	-0.35	-0.07	-0.13	0.13	0.28	0.24	0.48	0.25	0.30	0.27	0.03	-0.43	0.15	-0.23	0.43
Iamiaceae	0.73	0.17	0.52	0.23	0.34	0.43	0.24	0.37	0.67	0.44	0.33	0.43	0.43	0.44	0.43	0.49	0.67	-0.52	0.49	0.50	**0.90**	0.34	0.77	0.55	0.40
Kineosporiaceae	-0.10	0.42	-0.52	0.58	0.45	0.33	0.40	0.58	-0.57	0.27	0.33	0.32	-0.02	0.23	0.13	-0.32	-0.55	-0.35	-0.15	-0.02	0.03	0.58	-0.10	0.33	-0.50
Lachnospiraceae	-0.07	0.58	-0.38	0.54	0.39	0.38	0.47	0.58	-0.38	0.11	0.15	0.50	0.13	0.24	0.23	-0.28	-0.37	-0.28	-0.13	0.02	0.24	0.54	-0.03	0.27	-0.60
Methylobacteriaceae	-0.23	-0.47	-0.12	-0.49	0.03	0.02	-0.09	-0.03	-0.17	-0.23	0.00	-0.24	-0.58	0.11	-0.10	-0.08	-0.18	0.18	-0.30	-0.27	-0.63	-0.04	-0.37	-0.22	-0.10
Micrococcaceae	0.07	-0.65	0.37	-0.62	-0.19	-0.18	-0.22	-0.27	0.35	-0.24	-0.15	-0.07	-0.15	-0.08	-0.07	0.26	0.31	0.27	-0.09	-0.23	-0.26	-0.27	-0.23	-0.23	0.07
Micromonosporaceae	0.28	-0.05	0.35	0.06	0.45	0.10	0.49	0.53	0.18	0.06	0.53	0.04	0.10	0.23	-0.20	0.32	0.25	-0.38	-0.07	-0.08	-0.05	0.45	0.23	0.32	-0.05
Moraxellaceae	0.22	0.36	0.07	0.21	-0.20	-0.03	-0.11	-0.10	0.22	0.07	-0.23	0.29	0.43	-0.15	0.14	-0.02	0.20	0.00	0.15	0.12	0.69	-0.11	0.23	0.07	-0.02
Nocardioidaceae	-0.08	-0.08	-0.02	-0.21	-0.08	-0.02	-0.02	0.05	0.00	-0.28	-0.22	0.24	-0.07	-0.08	0.03	-0.06	-0.03	0.20	-0.30	-0.32	0.00	0.00	-0.32	-0.17	-0.40
Oscillospiraceae	0.07	-0.23	0.16	-0.68	-0.38	-0.07	-0.51	-0.40	0.37	-0.32	-0.38	-0.30	-0.41	-0.05	-0.01	0.04	0.33	0.33	-0.13	-0.08	0.12	-0.45	0.10	-0.31	0.37
Oxalobacteraceae	0.09	-0.56	-0.05	-0.32	-0.04	-0.29	-0.02	-0.07	-0.13	0.03	0.11	0.08	0.06	-0.14	-0.30	-0.12	-0.13	0.02	-0.21	-0.44	-0.34	-0.03	-0.38	0.03	-0.50
Pseudomonadaceae	-0.27	0.48	-0.07	-0.32	-0.60	-0.42	-0.40	-0.40	0.05	-0.68	-0.62	-0.43	-0.15	-0.51	-0.44	-0.28	0.08	0.50	-0.43	-0.33	0.21	-0.54	0.03	-0.53	0.03
Rhodobacteraceae	0.06	-0.79	0.18	-0.39	0.04	-0.16	-0.15	-0.08	0.06	0.07	0.23	-0.17	-0.24	-0.01	-0.13	0.24	0.03	0.05	-0.09	-0.24	-0.50	-0.04	-0.32	-0.02	0.10
Rubrobacteraceae	0.63	-0.26	0.55	0.02	0.53	0.34	0.46	0.59	0.51	0.30	0.54	0.47	0.28	0.45	0.21	0.54	0.52	-0.50	0.17	0.09	0.34	0.54	0.33	0.52	-0.02
Ruminococcaceae	-0.38	0.01	-0.69	-0.03	-0.27	-0.34	-0.28	-0.11	-0.69	-0.18	-0.24	0.00	-0.19	-0.44	-0.33	-0.63	-0.72	0.29	-0.61	-0.65	-0.25	-0.11	-0.69	-0.23	-0.74
Solirubrobacteraceae	0.33	-0.30	-0.03	0.31	0.66	0.68	0.42	0.55	-0.02	0.65	0.52	0.67	0.07	0.69	0.69	0.20	-0.07	-0.53	0.38	0.38	0.08	0.65	0.03	0.58	-0.17
Sphingobacteriaceae	-0.11	-0.50	-0.18	-0.57	-0.09	-0.09	-0.13	-0.12	-0.16	-0.22	-0.13	-0.01	-0.36	0.01	-0.14	-0.24	-0.19	0.20	-0.34	-0.41	-0.45	-0.11	-0.44	-0.18	-0.45
Sphingomonadaceae	0.73	-0.01	0.48	0.68	0.67	0.60	0.60	0.50	0.48	**0.93**	0.68	0.68	0.75	0.66	0.68	0.59	0.49	-0.82	**0.94**	**0.85**	0.61	0.62	0.70	**0.85**	0.33
Staphylococcaceae	-0.56	0.07	-0.65	-0.03	-0.39	-0.63	-0.26	-0.24	-0.78	-0.37	-0.28	-0.29	-0.16	-0.67	-0.66	-0.67	-0.76	0.41	-0.69	-0.77	-0.52	-0.26	-0.74	-0.39	-0.71
Veillonellaceae	-0.08	0.56	0.20	0.16	-0.24	-0.27	-0.09	-0.07	0.17	-0.33	-0.17	-0.35	0.12	-0.36	-0.32	0.11	0.22	0.15	-0.14	-0.05	0.32	-0.18	0.28	-0.20	0.35
Xanthomonadaceae	-0.05	0.17	-0.10	-0.02	-0.03	-0.07	0.00	0.22	-0.10	-0.27	-0.12	0.20	-0.03	-0.18	-0.12	-0.11	-0.11	0.12	-0.46	-0.43	0.18	0.12	-0.25	-0.08	-0.43

Values in bold are different from 0 with a significance level alpha=0.01

* Variables were stadardized (log_{10})

Figure 7 Spearman correlation matrix between the 40 most abundant bacterial families detected by HtS methods as the principal variable and geochemical attributes as the supplementary variable. All variables were transformed using log_{10} to approximate a normal distribution. Red shading represents significant negative correlations; shading in blue signifies positive correlations ($\alpha < 0.01$).

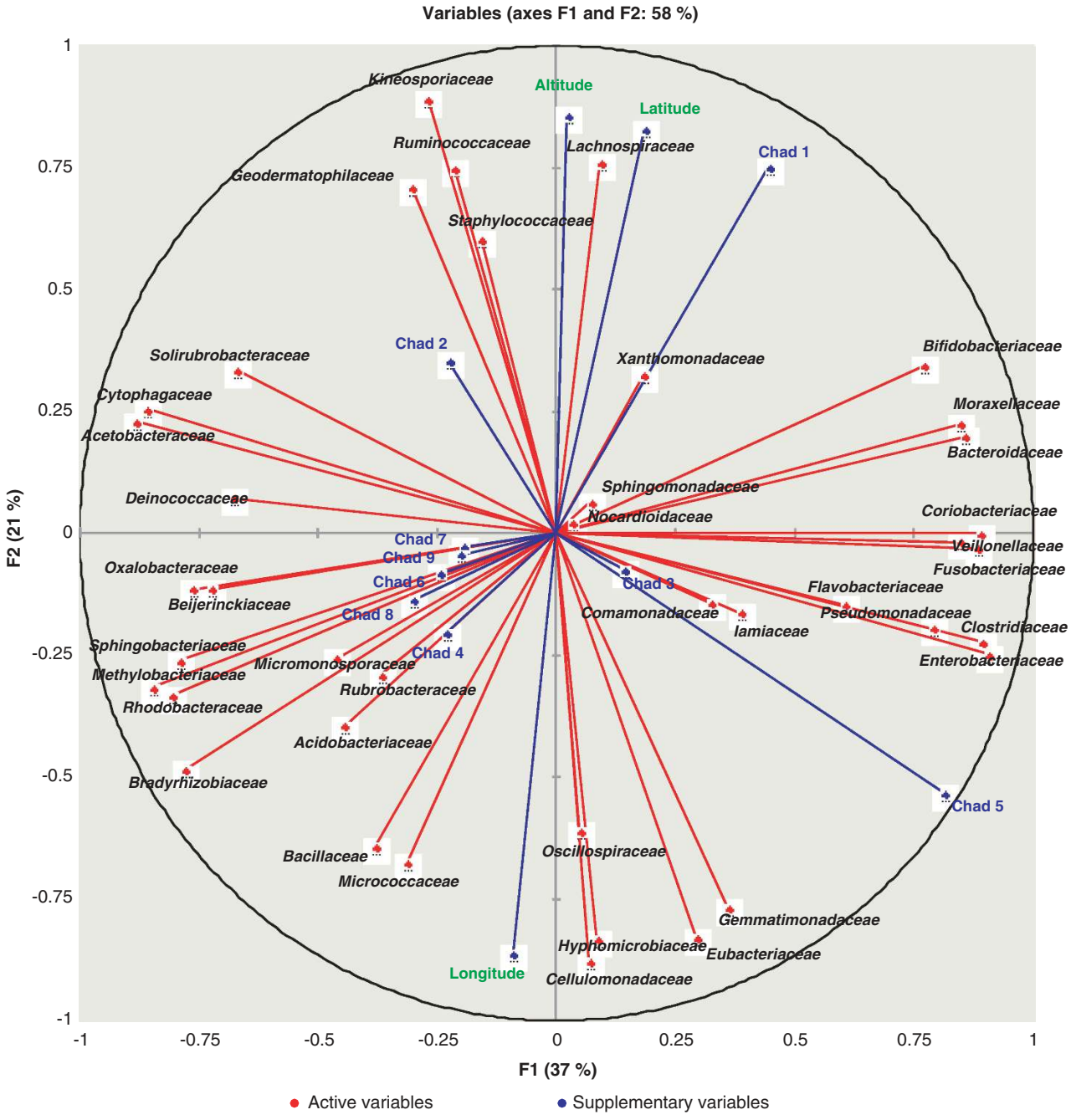


Figure 8 Principal component analyses were performed on the $\log_{10}$ transformed Spearman rank correlation matrices and used to cluster the 40 most abundant bacterial families found by HtS based methods with the geographical coordinates (altitude, latitude and longitude) of the places where samples C1–C9 were collected (see Table 1). Longer vectors indicate a larger contribution of the variable whereas the closer the variables are to one another, the higher the correlation between them.

and Crenarchaeota were present in the Tatouine along with most Proteobacterial groups (α, γ, δ and ε). Most probably the differences are not significant, but the diversity of Acidobacteria was greater in the Tatouine whereas more Firmicutes were found in Chad. As many Firmicutes readily form spores, this may imply higher efficiency of DNA extraction (Giongo *et al.*, 2012). Obviously, the greater depth of sequencing used here helped reveal more phyla in Chad—of the 59 genera identified by Chanal *et al.* (2006), only 19 were not found. Both analyses identified many bacteria that are resistant to radiation and/or desiccation.

Most concerns about the spread of human diseases revolve around the Proteobacterial family Enterobacteriaceae that contains the pathogenic genera *Enterobacter, Escherichia, Klebsiella, Salmonella, Serratia* and *Shigella*. Despite the proximity of a garrison near C1 and C2 or refugee camps near C5, none of these species were abundant (<0.1% total

reads). Traces of *Erwinia* spp. were present in C4, C6, C7, C8 and C9. Enterobacteriaceae were presumably of human origin and therefore not able to survive long as they are strangers to the desert niche as well as inherently poor survivors in sand (see Toepfer *et al.*, (2012)). In any event, most introduced microorganisms quickly succumb to competition with normal soil inhabitants and may also be engulfed by protozoa (Prescott *et al.*, 2003).

Most mycobacteria are non-pathogenic, soil bacteria from which infectious species such as *M. tuberculosis* and *M. leprae* probably evolved. It is thus neither alarming nor surprising that *Mycobacterium* spp. were found in almost all samples (frequency ~0.1%). Similarly, environmental genera that contain opportunistic pathogens were present including *Alcaligenes, Brevundimonas, Massilia, Nocardiopsis* and *Sphingomonas.* Other environmental genera including *Aurantimonas, Clavibacter, Leifsonia, Sphingomonas* and *Xanthomonas*, that despite taxonomic uncertainties, may include potential plant pathogens, were also present. All members of the family Bacillaceae form endospores and some of them are animal pathogens. Since HtS methods did not permit assigning bacteria to a species, it is possible that *B. anthracis* was present in some of the samples.

Obvious environmental adaptations included various pigments that protect the desert consortia from: (a) solar radiation; (b) high temperatures including a number of Actinobacteria—*Pseudonocardia* (Pseudonocardiaceae) and *Streptomyces* (Streptomycetaceae); Thermomonosporaceae as well as other groups like *Alterococcus, Nitrospira, Porphyrobacter* and *Rubellimicrobium*. The Geobacteraceae includes both thermophilic and psychrophilic species. *Geobacter stearothermophilus* is even used to check autoclaves; (c) γ radiation especially in *Deinococcus, Hymenobacter, Kineococcus, Methylobacterium* and *Rubrobacter*; (d) desiccation (often associated with resistance to radiation) for example, in *Arthrobacter, Candidatus-Solibacter, Corallococcus, Kineococcus* and *Methylobacterium*; (e) psychrotolerance (a characteristic of Antarctic bacteria but also necessary at night in the Sahara) in *Frigobacterium, Planococcus, Subtercola,* some Rhodobacteraceae (Antarctic inhabitants), *Modestobacter* (also Antarctic) (Hirsch *et al.*, 2004a, b) and (f) halophyly in *Alterococcus, Salinarimonas,* and *Nocardioides* (*N. halotolerans* survives 1.7 M NaCl). Developmental adaptations (for example, spore formation) are prevalent in the Bacillaceae/Paenibacillaceae: for this reason they are widespread, diverse and numerous. Indeed, *Bacillus subtilis* is ecumenical in the sands and dust of Chad.

Cytophagaceae, a family that contains many marine bacteria (often associated with fish such as *Flexibacter* or *Flectobacillus*) were abundant. Some marine Actinobacteria (*Salinispora* and *Verrucosispora*) were also present. Other species linked to aquatic life, some of them motile including: (a) Actinobacteria—*Cryptosporangium*, *Nocardioides* and *Sporichthya* (motile spores); (b) Proteobacteria—*Chelatococcus* (Beijerinckiaceae), Haliangiaceae, some *Rhodoplanes* (Hyphomicrobiaceae), *Roseomonas* (Acetobacteraceae) and *Skermanella* (Rhodospirillaceae); (c) many Rhodocyclaceae, Rhodospirillaceae and Sphingomonadaceae, and (d) Planctomycetaceae were also found. It is not clear whether these aquatic, mobile species adapted to desert conditions including higher salt concentrations as Mega-Lake Chad dried out or are special desert forms of aquatic relatives, because HtS-based techniques do not yield a living organism for further study. Motile bacteria that associate with the roots of plants like *Herbaspirillum* and *Mesorhizobium* (respectively β- and α-Proteobacteria) were also present.

The oligotrophic families Geodermatophilaceae, Pseudonocardiaceae, Rhodocyclaceae and Rubrobacteraceae were particularly abundant. Most bacteria found were aerobic but strict anaerobes including members of the Clostridiaceae, Fusobacteriaceae, Geobacteraceae, Lactobacillaceae, Oxalobacteraceae, Opitutaceae and Rhodospirillaceae were present. Facultative anaerobes including the Enterobacteriaceae, Myxococcaceae, some Opitutaceae and Rhodospirillaceae were found along with *Rhodomicrobium*.

A few thermophilic and anaerobic methanogens (Euryarchaeota) belonging to the family Methanothermaceae (Methanobacteriales) were found especially in C1 and C2. The proteobacterial family Methylobacteriaceae (Rhizobiales) includes numerous *Methylobacterium* and *Microvirga*: some of the latter can reduce nitrogen gas to ammonia (Ardley *et al.*, 2012). Methanol and other carbon compounds are used for growth. Methanotrophic Methylocystaceae (α-Proteobacteria, Rhizobiales) that obtain energy from methane and fix nitrogen were found. Many other bacteria that can fix nitrogen under free-living conditions or in association with plants including Beijerinckiaceae, Bradyrhizobiaceae and Rhizobiaceae (Rhizobiales), some *Mesorhizobium* (Phyllobacteraceae), *Azospirillum* and *Rhodospirillum* (Rhodospirillaceae), *Frankia* (Actinobacteria, Frankiaceae) and *Herbaspirillum* (Oxalobacteraceae) were present. In general the Beijerinckiaceae fix nitrogen under free-living conditions whereas *Bradyrhizobium, Frankia*, *Mesorhizobium* and *Rhizobium* are symbionts of plants. This abundance of nitrogen-fixing bacteria highlights the scarcity of nitrogenous compounds in the deserts of Chad.

Heterotrophy was not the only life style observed. Members of the families Desulfurococcaceae and Sulfolobaceae (Crenarchaeota) are normally thermophilic and use sulphur either for anaerobic respiration or as a source of energy (Desulfurococcaceae). Photoheterotrophic α-Proteobacteria that require organic carbon under anoxic conditions in light include *Blastochloris*, *Rhodomicrobium, Rhodoplanes* (all Hyphomicrobiaceae) and some

Rhodobacteraceae. Other families containing photoheterotrophic bacteria include the Rhodobiaceae, Rhodocyclaceae and Rhodospirillaceae. Bacteria that can be photoheterotrophic under aerobic conditions included *Paracraurococcus*, *Porphyrobacter* and some *Sphingomonas* (all Proteobacteria).

That biodegradation is an important aspect of microbial life in deserts is shown by the abundance of Actinobacteria, many of which are capable of efficiently hydrolysing cellulose and lignin (for example, *Cellulosimicrobium* and *Isoptericola*). Other bacteria able to digest plant material were found (Cystobacteraceae, Cytophagaceae and Polyangiaceae) along with many others that can degrade complex organic compounds including synthetic molecules.

Members of Geodermathophilaceae were described from desert rocks and soils (Hungate *et al.*, 1987; Eppard *et al.*, 1996; Garrity *et al.*, 1996); they are also associated with degradation of carbonaceous monuments (Urzi and Realini, 1998; Urzì *et al.,* 2001). Black pigmented Geodermatophilaceae show high stress resistances, including to massive doses of γ radiation (30 kGy) that is comparable to levels tolerated by *Deinococcus radiodurans* (Rainey *et al.*, 1995).

Fungal isolates represent a random collection of mostly dark pigmented spore-forming fungi. With the exception of *Cochliobolus lunatus*, which was frequently present, most isolates were found in only one sample. Data from HtS reflect the relatively poor representation of fungi in molecular data databases. No doubt fungal spores can travel with dust clouds and have a role in respiratory illness. However, all attempts to isolate rock-inhabiting and/or desiccation-tolerant fungi (such as microcolonial fungi *sensu* Staley *et al.*, 1982) delivered only small black colonies of the bacterium *Geodermatophilus obscurus*.

All algal isolates belonged to the class Trebouxiophyceae (Figure 5), well known for its terrestrial members (Friedl, 1995). *Ha. pluvialis* produces astaxanthin that protects the photosynthetic apparatus from photo-oxidative damage (under high light intensities) or nutrient stresses (Vidhyavathi *et al.,* 2008). HtS showed that members of the Ulvophyceae, Chlorophyceae, Charophyceae, Prasinophyceae, Zygnemophyceae, Bacillariophyceae and Chrysophyceae were also present, but they may have been dormant and thus not observed in culture.

The microbial catalogue of dust collected onboard the 'Stad Amsterdam' in the Cape Verde Islands was similar to that found in the sands of Chad. In contrast, dust from the Bodélé Depression had fewer Bacilli. Part of this difference is undoubtedly traceable to varying soil types—the Bodélé Depression was once part of Mega-Lake Chad. As a consequence, the lake sediments are rich in calcium whereas the desert sands are siliceous. Furthermore, it is conceivable that constant erosion in the Bodélé Depression restricts the establishment of some bacterial populations and blows away bacterial spores. Nevertheless with only one exception, all bacteria isolated from the Bodélé Depression were also found using HtS methods in other parts of Chad. This strongly suggests that the microbial populations are similar but that saltation alters their occurrence.

Shinn *et al.* (2000) suggested that dust from Africa carried *Aspergillus sydowii* to the Caribbean where it caused the demise of coral reefs. We showed that *A. sydowii* remains viable even after extended storage periods on dry sand (Toepfer *et al.*, 2012). However, even though we found *A flavus* and *A. niger* in two samples of Chad sand, *A. sydowii* was not present (Table 7). Furthermore, *Aspergillus* spp. were not present in the dust collected aboard the 'Stad Amsterdam'. Rypien (2008) collected air samples in Mali during three dust storms (as well as one in St. Croix (Caribbean)) and isolated several *Aspergillus* spp. but not *A. sydowii*. Absence of *A. sydowii* is not proof that it does not occur in Africa nor should it be forgotten that dust clouds are dynamic ecosystems that can gain and lose hitchhikers as they pass over land and sea (Griffin, 2007). In other words, our results primarily describe the microbial load at the point of departure but they do not measure changes that may occur as the dust clouds move around the planet.

In summary, most hitchhikers on desert dust are environmental microbes that are well adapted to the harsh conditions. These same adaptations help them survive intercontinental travel. If contamination occurs at the source, pathogens can accompany the desert dwellers but are less likely to survive the journey.

Conflict of Interest

The authors declare no conflict of interest.

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