

## 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 × 10<sup>6</sup> 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; Kellogg *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 (Bahai 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 <sup>a</sup>	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 Bahai Wadi I	Sandman B	30.04.07	N15.37.473	E23.00.626	781	Clumped, red-brown sand
C4 Bahai 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.

<sup>a</sup>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 Bahai-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 <i>et al.</i> (2008)	10.03.05 to 12.03.05	N16.52.53	E18.32.548	179 m	surface
Wp 45 Bodélé near Chica	see Chappell <i>et al.</i> (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 <i>et al.</i> (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 toilettes 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.

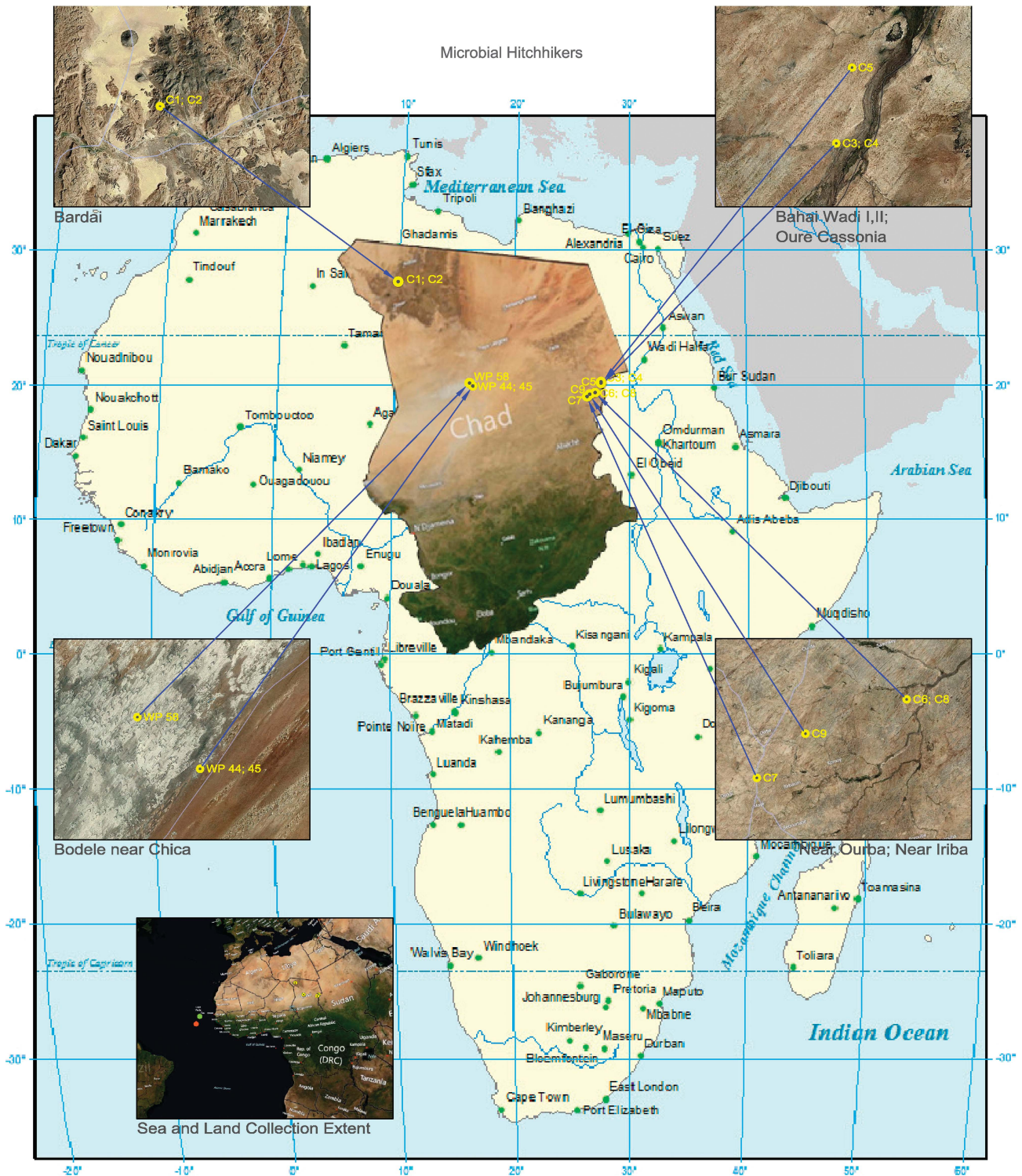
analyses (using XLSTAT2011 software) performed on the transformed correlation matrices (Addinsoft, New York, NY, USA).

## Results

### Geochemistry

Sand was analysed for both major and trace elements (see Figure 2a, b). Dust from the Bodélé

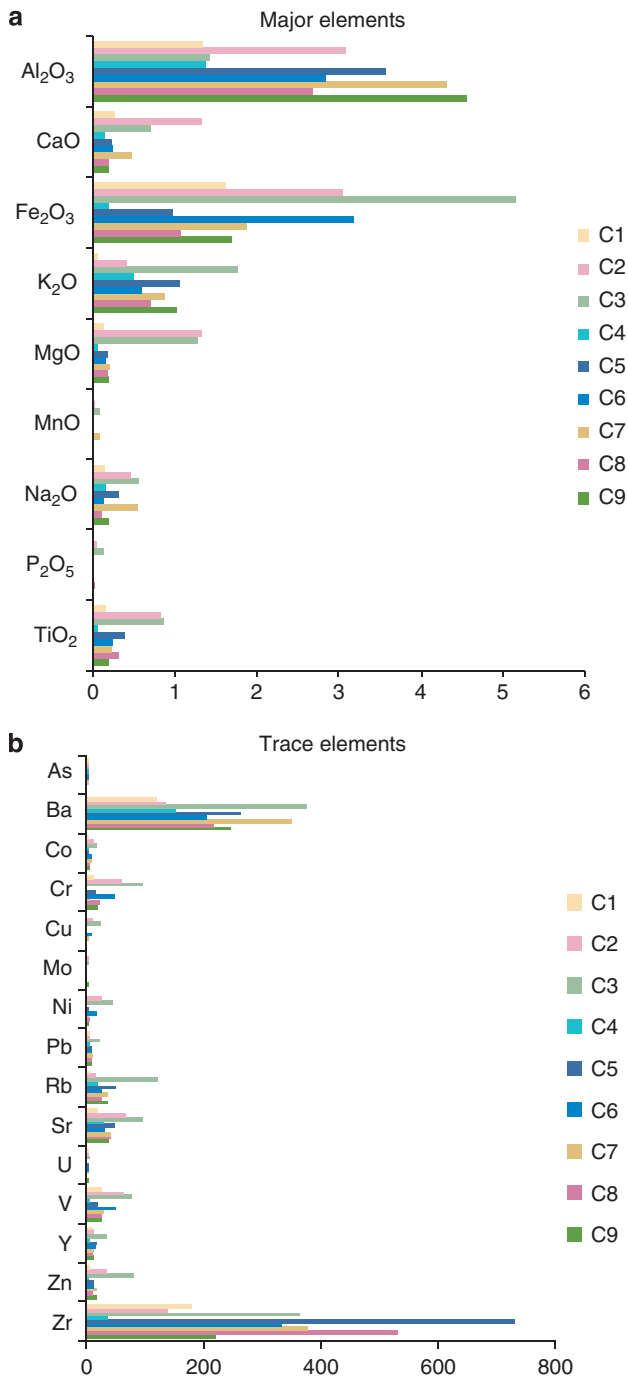
Depression was only analysed for major elements. With the exception of C3 (64 weight (wt) %), all sand samples contained at least 85 wt % SiO<sub>2</sub> (in wt % respectively: C1 93; C2 85; C4 95; C5 89; C6 87; C7 87; C8 92 and C9 89). Mineral contents varied widely and without association with location (for example, Fe). Sample C4 contained 0.2 wt % Fe<sub>2</sub>O<sub>3</sub>, 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  $\text{mg kg}^{-1}$ .

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

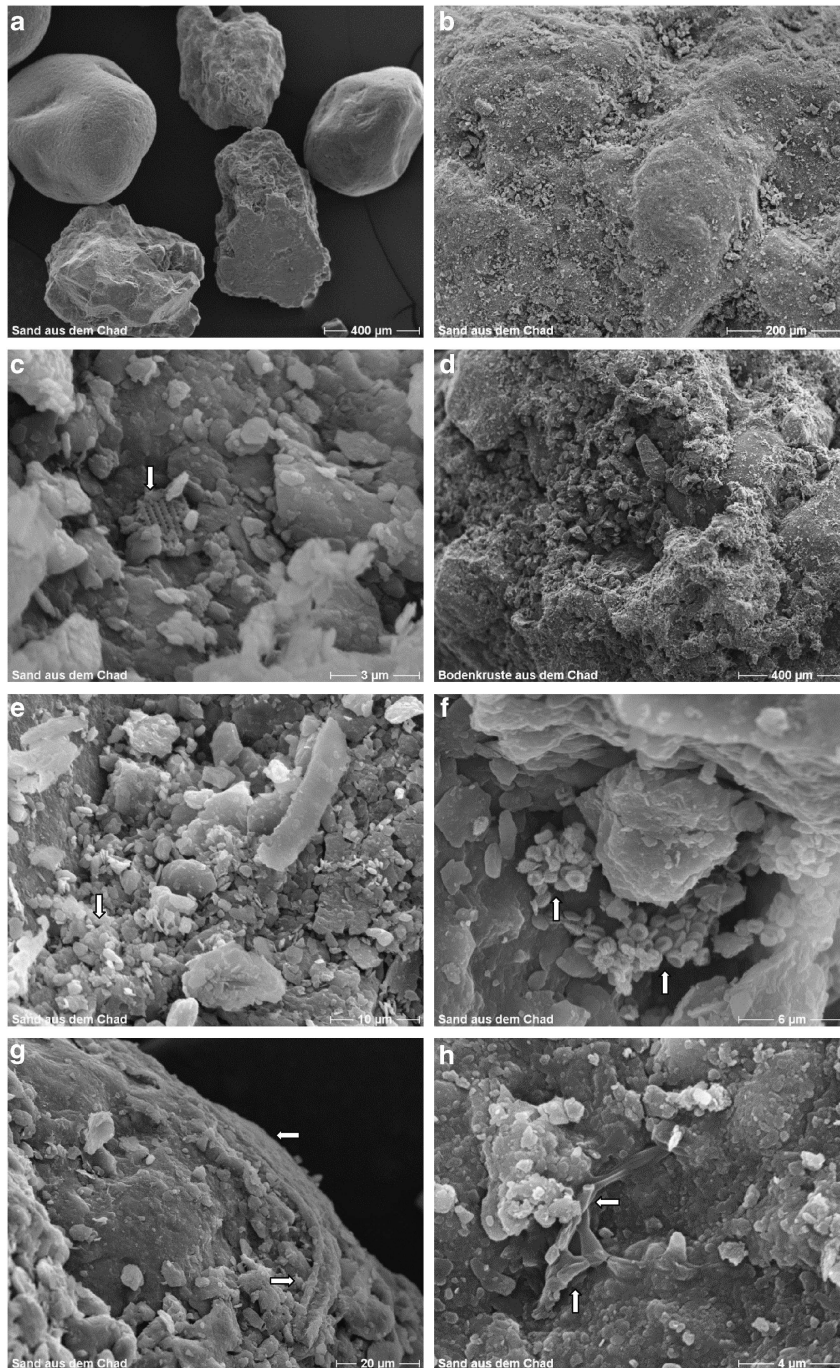
SEM showed, with the exception of C3, that the non-hydrated samples were a collection of loose grains to which small particles adhered (Figure 3). Under these *in situ* conditions, it was difficult to distinguish microbes from particles of clay, diatom fragments and other adhering material. Nevertheless, 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  $\geq 0.3\%$  of total reads and the seven most abundant families are shown in

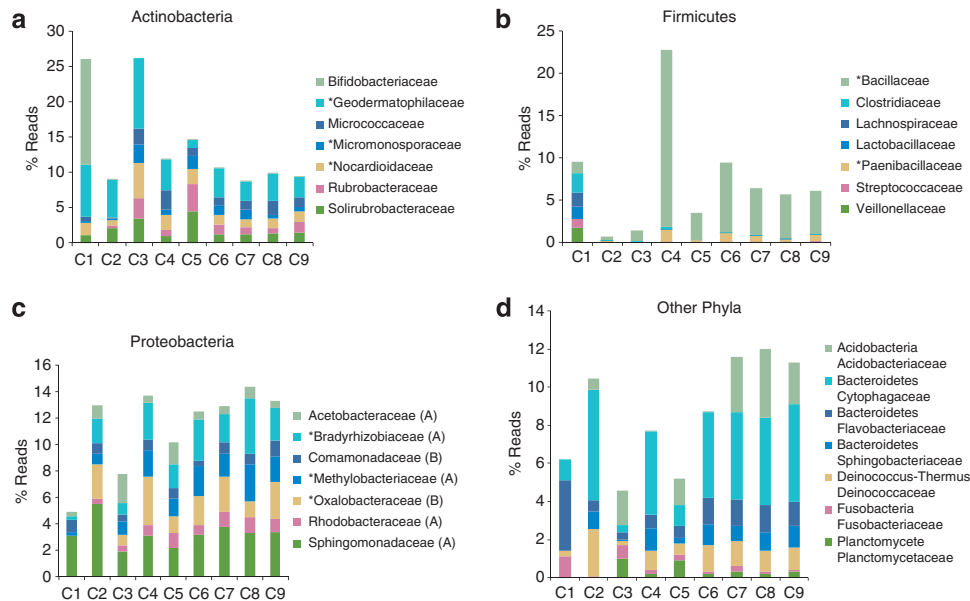


**Figure 3** SEM micrographs of air-dried samples of surface material from Chad: Panel (a) = C2; (b) = C1; (c) = C1 magnified; (d) = C3; (e) = C4; (f) = C5; (g) = C6 and (h) = C5. Except for C3, where sand particles were held together by a thick layer of biological material, all other samples were collections 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 Archaea – 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  $\geq 0.5\%$  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  $\geq 0.3\%$  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  $\geq 0.5\%$  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  $\geq 0.3\%$  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  $\geq 0.3\%$  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 (*Mucilaginitacter*, *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  $\geq 1\%$  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:  $\alpha$ -Proteobacteria (22 families);  $\beta$ -Proteobacteria (11 families);  $\gamma$ -Proteobacteria (18 families);  $\delta$ -Proteobacteria (17 families);  $\varepsilon$ -Proteobacteria (2 families), Campylobacteraceae (traces in C8 and C9), Helicobacteraceae (traces in C8) and  $\zeta$ -Proteobacteria (traces of Mariprofundaceae in C6).

Some families were abundant in almost every sample: Bradyrhizobiaceae, Methylobacteriaceae and

Sphingomonadaceae ( $\alpha$ -Proteobacteria) and Oxalobacteraceae ( $\beta$ -Proteobacteria) for example. Other families were less abundant but well distributed. Less numerous families ( $< 0.3\%$ ) included: Bacteriovoraceae ( $\delta$ ); Bdellovibrionaceae ( $\delta$ ); Coxiellaceae ( $\gamma$ ); Erythrobacteriaceae ( $\alpha$ ); Legionellaceae ( $\gamma$ ); Methylocystaceae ( $\alpha$ ); Methylophilaceae ( $\beta$ ); Neisseriaceae ( $\beta$ ); Nitrosomonadaceae ( $\beta$ ); Pasteurellaceae ( $\gamma$ ); Phaselicystidaceae ( $\delta$ ); Phyllobacteriaceae ( $\alpha$ ); Polyangiaceae ( $\alpha$ ); Rhodobiaceae ( $\alpha$ ); Rhodocyclaceae ( $\beta$ ); Rickettsiaceae ( $\alpha$ ); Sinobacteriaceae ( $\gamma$ ) and Xanthobacteraceae ( $\alpha$ ).

#### 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). Acido-

(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* (*Micrococcaeae*); *Nocardioidea* (*Nocardioideaceae*); *Rubrobacter* (*Rubrobacteraceae*) and *Solirubrobacter*

(*Solirubrobacteraceae*) were the principal genera found. Other well-distributed genera included: *Actinoplanes*, *Cellulomonas*, *Conexibacter*, *Modes-tobacter*, *Iamia*, *Kineococcus*, *Kineosporia*, *Kocuria*, *Marmoricola*, *Micromonospora*, *Pseudonocardia* and *Streptomyces*.

*Bacillus* was the principal genus of the Firmi-cutes (% 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* (*Beijerinck-iaceae*), *Belnapia*, *Bosea*, *Bradyrhizobium*, *Burkhol-deria*, *Chelatococcus*, *Cystobacter*, *Duganella*, *Herbaspirillum*, *Janthinobacterium*, *Kaistobacter*, *Massilia* (*Oxalobacteraceae*), *Rhodmicrobium*, *Rubellimicrobium*, *Sinorhizobium*, *Sphingosinicella* and *Variovorax* (various  $\alpha$ - and  $\beta$ -*Proteobacteria*).

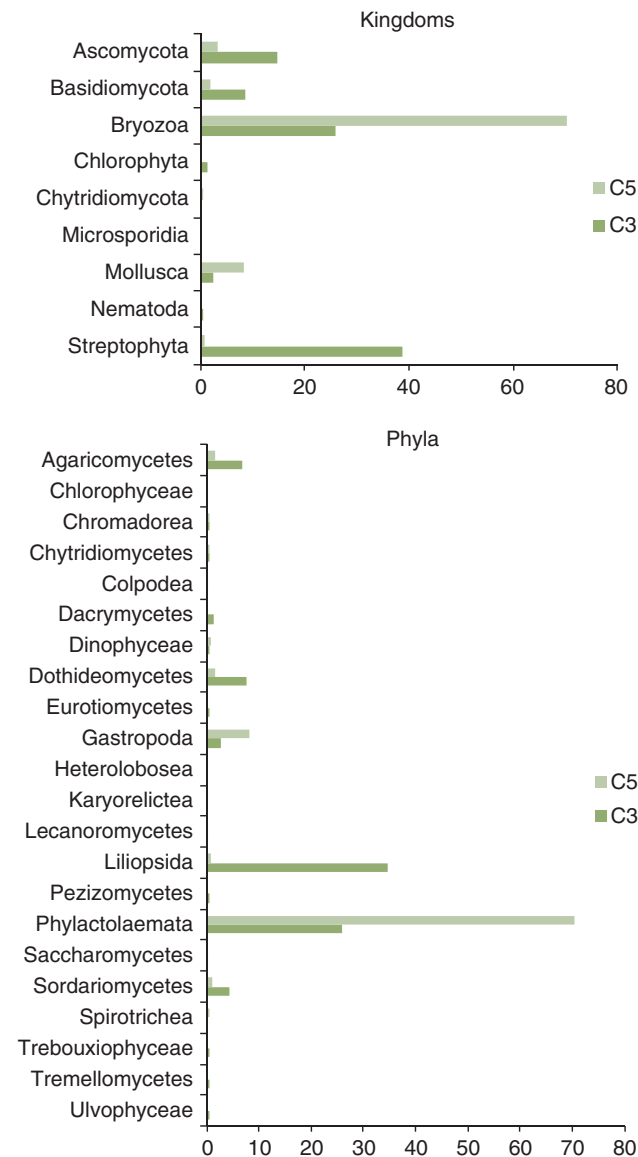
Other phyla that contained named genera included Bacteroidetes with *Bacteroidaceae* (*Bacteroides*) and *Cytophagaceae* (*Adhaeribacter*, *Flexibacter*, *Hymenobacter* and *Sporocytophaga*). Less abundant genera in the *Flavobacteriaceae* and *Sphingobacteriaceae* as well as *Deino-coccus* (*Deinococcus-Thermus*) and *Nitrospira* (*Nitrospirae*) were also present. Other well-distributed genera included *Acidimicrobia* (*Cyclo-bacteriaceae*), *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 phytopatho-gen *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, Micro-sporidia and Glomeromycetes were also present. Bryozoa (aquatic, invertebrate animals) and Strepto-phyta (land plants and green algae) were also prevalent. Mollusca, which includes terrestrial repre-sentatives, 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



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



(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* (Maciá-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  $\geq 0.01\%$  of total ‘reads’ in the nine samples of Chad sand

Phylum	Number of: Families	Genera	Number of reads: 0.1–0.9%	$\geq 1\%$	$\geq 1\%$ 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

	C1	C2	C3	# Bacteria (CFU g <sup>-1</sup> ) C4	C5	C6	C7	C8	C9
Total # OTU	$9 \times 10^2$	$5.4 \times 10^3$	$2.2 \times 10^5$	$7.4 \times 10^4$	$2.2 \times 10^4$	$1.5 \times 10^5$	$1.2 \times 10^5$	$3.6 \times 10^5$	$5.4 \times 10^5$
# Total spores	$2.8 \times 10^1$	$2.8 \times 10^1$	$2.2 \times 10^5$	$2 \times 10^4$	$6.2 \times 10^3$	$1.2 \times 10^3$	$7 \times 10^2$	$1.9 \times 10^3$	$5.6 \times 10^2$
% 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
<sup>a</sup> <i>Agrobacterium tumefaciens</i>	C4		
<i>Arthrobacter dextranolyticus</i>	C7		
<i>A. globiformis</i>	C2		
<i>Arthrobacter</i> spp.	C2, C7, C9	WP44	
<sup>a</sup> <i>Azospirillum</i> spp.	C7		
<i>Bacillus badius</i>			F3,A
<i>B. casamensis</i>	C2		
<i>B. circulans</i>	C6		
<i>B. drementensis</i>	C3		
<i>B. endophiticus</i>	C4		F3, F4
<i>B. firmus</i>			F7
<i>B. foraminis</i>	C1, C2		
<i>B. funiculus</i>	C6		
<i>B. horikoshii</i>			F4
<i>B. massiliensis</i>	C7		
<i>B. megaterium</i>	C4, C5, C6, C7, C8		
<i>B. nearlsonii</i>			F1, A, B, M
<i>B. niabensis</i>			F4
<i>B. niacini</i>			D
<i>B. pocheonensis</i>	C7		
<i>B. pumilus</i>	C3		F2,F4
<i>B. subtilis</i>	C2, C3, C4, C5, C6, C7, C8, C9	WP44	F1, F2, F4, A, B, M
<i>B. thuringiensis</i>	C3		
<i>Bacillus</i> spp.	C2, C3, C5, C6, C7, C8		
<i>Blastococcus</i> spp.		WP44, WP58	
<sup>a</sup> <i>Bradyrhizobium</i> spp.	C4, C5, C7	WP58	
<i>Brevibacillus borstelensis</i>	C1		
<i>Chelatococcus ascharovorans</i>	C9		
<sup>a</sup> <i>Cohnella</i> spp.	C5		
<i>Cutobacterium citreum</i>	C3		
<i>Curtobacterium</i> spp.	C6, C7		
<i>Duganella violaceusniger</i>	C4, C5, C7		
<i>Exiguobacterium</i> spp.	C5, C9		
<i>Geodermatophilus obscurus</i>	C5		
<i>Geodermatophilus</i> spp.	C9		
<i>Hymenobacter</i> spp.	C7		
<i>Kocuria</i> spp.	C1	WP45, WP58	F4
<i>Massilia timonae</i>		WP45	
<i>Micromonospora</i> spp.		WP58	D
<i>Microvirga</i> spp.			D
<i>Neisseria</i> spp.			
<i>Nitrospira multiformis</i>	C7, C8		
<i>Nitrospira</i> spp.	C4, C6		
<i>Nocardioides kribbensis</i>		WP45	
<i>Oceanobacillus picturae</i>	C4		
<i>Ornithimicrobium humiphilum</i>		WP58	
<i>Oxalobacteraceae</i>	C7		F4
<i>Oxalobacter</i> spp.	C4		
<i>Paenibacillus macerans</i>			A
<sup>a</sup> <i>P. phyllosphaerae</i>	C4		
<i>P. validus</i>			F7
<i>Paenibacillus</i> spp.	C8		

**Table 6** (Continued)

Bacteria	Chad sand	Bodélé dust	'Beagle' dust
<i>Planococcus</i> spp.	C6		
<i>Pontibacter</i> spp.	C9		
<i>Pseudomonas luteola</i>	C5		
<sup>a</sup> <i>Rhizobium</i> spp.	C7		
<i>Rothia</i> spp.	C1		
<i>Shigella/Escherichia</i>		WP44	
<i>Sinorhizobium</i> spp.	C5		
<i>Sphingomonas yabuuchaie</i>	C3		
<i>Sporosarcina</i> spp.	C6		
<i>Streptomyces diastaticus</i>	C3		
<i>S. djakartensis</i>	C2		
<i>S. lilaceus</i>	C7		
<i>S. pseudogriseolus</i>	C5		
<i>S. purpurescens</i>	C5		
<i>S. rochei</i>	C3		
<i>S. violaceoruber</i>	C3		
<i>Terribacillus halophilus</i>			F3
<i>T. shanxiensis</i>	C8		
<i>Tsukamurella</i> spp.		WP45, WP58	
<i>Virgibacillus picturae</i>	C4		

For abbreviations see Tables 1 to 3.

<sup>a</sup>'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 pluvialis* 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)
<i>Ampelomyces</i> sp.	C9	Leptosphaeriaceae; Pleosporales, Ascomycota
<i>Aspergillus flavus</i>	C3	mitosporic Trichocomaceae, Eurotiales, Ascomycota
<i>Aspergillus niger</i>	C8	mitosporic Trichocomaceae, Eurotiales, Ascomycota
<i>Capnobotryella</i> sp.	C2	Teratosphaeriaceae, Capnodiales, Ascomycota
<i>Cochliobolus lunatus</i>	C3, C4, C7, C8, C9	Pleosporaceae, Pleosporales, Ascomycota
<i>Eurotium chevalieri/amstelodamii</i>	C8	Trichocomaceae, Eurotiales, Ascomycota
<i>Exserohilum rostratum</i>	C9	Pleosporaceae, Pleosporales, Ascomycota
<i>Fusarium equiseti</i>	C3	Mitosporic Hypocreales, Ascomycota
<i>Fusarium</i> sp.	C3, C9	Mitosporic Hypocreales, Ascomycota
<i>Humicola fuscoatra</i>	C9	Mitosporic Ascomycota
<i>Myrothecium verrucaria</i>	C8	Mitosporic Hypocreales, Ascomycota
<i>Phoma macrostoma</i>	C8	Mitosporic Ascomycota
<i>Pleiochaeta ghindensis</i>	C9	Mitosporic Pezizomycotina, Ascomycota
<i>Teratosphaeria</i> sp.	C2	Teratosphaeriaceae, Capnodiales, Ascomycota
<i>Trichoderma inhamatum</i>	C3	Hypocreaceae, Hypocreales, Ascomycota
<i>Ulocladium</i> sp.	C5	Pleosporaceae, Pleosporales, Ascomycota
<i>Westerdykella nigra</i>	C7	Sporangiaceae, Pleosporales, Ascomycota
Species from 'Beagle' dust	Sample	Taxonomic position
<i>Alternaria alternata</i>	F2	Pleosporales, Pleosporaceae, Ascomycota
<i>Bipolaris spicifera</i>	F2	Pleosporales, Pleosporaceae, Ascomycota
<i>Bipolaris spicifera</i> or <i>Cochliobolus australiensis</i>	F3, F4	Pleosporaceae, Pleosporales, Ascomycota
<i>Cladosporium sphaerospermum</i> or <i>Cladosporium lignicola</i>	F4, F4	Davidiellaceae, Capnodiales, Ascomycota
<i>Cochliobolus lunatus</i> or <i>Macrophomina phaseolina</i>	F1, F2, F4, F4	Pleosporaceae, Pleosporales, Ascomycota or Botryosphaeriaceae, Botryosphaeriales, Ascomycota
<i>Emericella nidulans</i> or <i>Emericella quadrilineata</i>	F4	Trichocomaceae, Eurotiales, Ascomycota
<i>Phoma betae</i> or <i>Macrophoma</i> sp.	F2	Mitosporic Ascomycota
<i>Phoma</i> sp.	F2	Mitosporic Ascomycota
<i>Thielavia arenaria</i> or <i>Thielavia subthermophila</i>	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—Bacillariophyceae (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<sub>2</sub> and

Zn (Figure 7). On the other hand, the sixth most abundant family, Geodermatophilaceae, was negatively correlated with the presence of Ba, K<sub>2</sub>O, Pb and Rb. This contrasts with the Gemmatimonadaceae 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

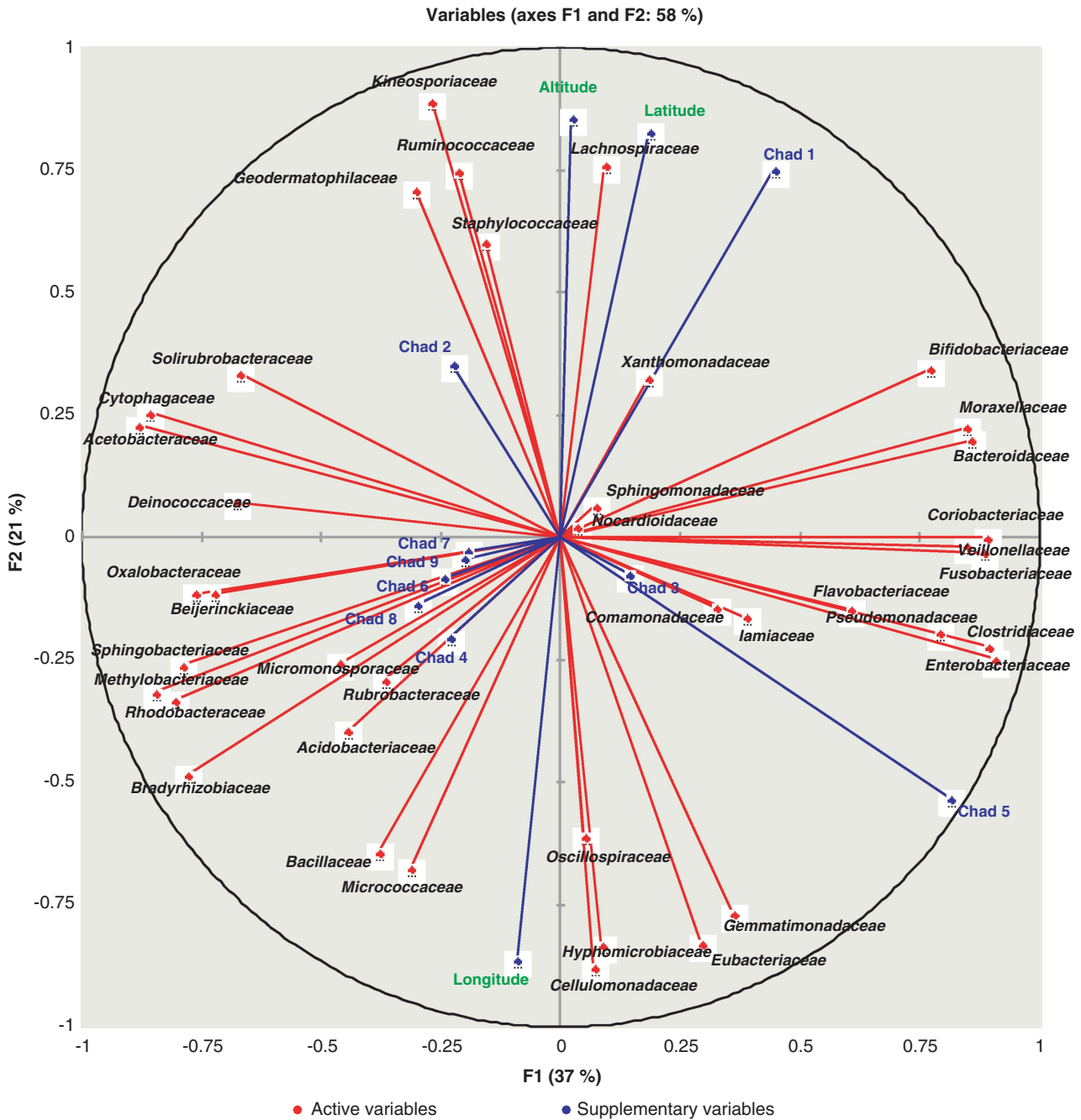
Variables*	Bacterial Families																																										
	Altitude	Latitude	Longitude	Acetobacteraceae	Acidobacteriaceae	Bacillaceae	Bacteroidaceae	Beijerinckiaceae	Bifidobacteriaceae	Bradyrhizobiaceae	Cellulomonadaceae	Clostridiaceae	Comamonadaceae	Coriobacteriaceae	Cytophagaceae	Deinococcaceae	Enterobacteriaceae	Eubacteriaceae	Flavobacteriaceae	Fusobacteriaceae	Gemmatimonadaceae	Geodermatophilaceae	Geodermatophilaceae	Hyphomicrobiaceae	Iamiaceae	Kineosporiaceae	Lachnospiraceae	Methylobacteriaceae	Micromonosporaceae	Moraxellaceae	Nocardioidaceae	Oscillospiraceae	Oxalobacteraceae	Pseudomonadaceae	Rhodobacteraceae	Rubrobacteraceae	Ruminococcaceae	Solirubrobacteraceae	Sphingobacteriaceae	Sphingomonadaceae	Staphylococcaceae	Veillonellaceae	Xanthomonadaceae
Altitude	1.00	0.16	<b>0.85</b>	0.41	-0.35	-0.46	0.18	-0.10	0.05	-0.21	-0.73	-0.44	0.24	-0.18	0.48	0.39	-0.40	<b>-0.89</b>	0.14	-0.24	-0.69	0.53	-0.78	-0.10	0.80	0.32	-0.14	-0.70	-0.26	-0.01	-0.38	-0.36	-0.02	-0.38	0.01	-0.37	0.70	-0.22	0.11	0.54	-0.16	-0.03	
Latitude	0.16	1.00	-0.10	-0.25	-0.70	-0.71	0.34	-0.31	0.72	-0.65	-0.30	0.34	-0.10	0.25	-0.38	-0.35	0.18	-0.10	-0.10	0.23	-0.28	0.38	-0.06	0.03	0.21	0.44	-0.65	-0.54	-0.72	0.67	0.00	-0.23	-0.46	0.33	-0.81	-0.62	-0.22	-0.14	-0.45	0.15	0.14	0.21	0.03
Longitude	<b>-0.95</b>	-0.10	1.00	-0.49	0.18	0.40	-0.01	-0.02	0.05	0.16	<b>0.83</b>	0.52	-0.03	0.26	-0.57	0.34	0.48	<b>0.94</b>	0.08	0.25	0.76	-0.65	<b>0.97</b>	0.27	<b>-0.87</b>	-0.49	0.01	0.53	0.08	0.13	0.13	0.54	-0.33	0.48	0.11	0.18	-0.78	-0.47	0.06	-0.03	-0.65	0.24	-0.18
Acetobacteraceae	0.41	-0.25	-0.49	1.00	0.23	0.18	-0.73	0.70	-0.70	0.63	-0.13	-0.80	-0.35	-0.78	0.77	0.73	<b>-0.88</b>	-0.57	-0.43	-0.75	-0.57	0.53	-0.25	-0.48	0.43	-0.03	0.68	-0.03	0.27	0.82	-0.28	-0.21	0.45	-0.77	0.60	0.00	0.23	0.58	0.50	-0.02	0.24	-0.73	-0.37
Acidobacteriaceae	-0.35	-0.70	0.18	0.23	1.00	0.33	-0.33	0.61	-0.54	0.40	0.07	-0.29	-0.46	-0.26	0.28	0.11	-0.15	0.18	-0.24	-0.10	0.27	0.35	0.13	-0.08	-0.16	-0.05	0.53	0.63	0.71	-0.58	0.35	-0.18	0.19	-0.51	0.60	0.76	-0.47	0.40	0.26	0.14	-0.40	-0.19	0.15
Bacillaceae	-0.46	-0.71	0.40	0.18	0.33	1.00	-0.60	0.15	-0.68	0.75	0.68	-0.02	0.00	-0.12	0.03	0.13	-0.07	0.25	0.00	-0.12	0.25	-0.08	0.27	-0.33	-0.45	-0.50	0.70	0.65	0.63	-0.63	0.12	0.36	0.55	0.12	0.60	0.35	-0.18	-0.33	0.65	-0.60	0.09	-0.12	-0.03
Bacteroidaceae	0.18	0.34	-0.01	-0.73	-0.33	-0.60	1.00	-0.68	0.72	<b>-0.85</b>	-0.23	0.57	0.42	0.72	-0.50	-0.37	0.73	0.08	0.60	0.65	0.30	-0.40	-0.17	0.60	0.02	0.15	<b>-0.90</b>	-0.53	-0.42	-0.82	-0.18	-0.06	-0.68	-0.43	-0.68	-0.24	-0.12	-0.32	<b>-0.88</b>	0.42	-0.20	0.72	0.10
Beijerinckiaceae	-0.10	-0.31	-0.02	0.70	0.61	-0.68	1.00	-0.46	0.65	0.00	-0.63	-0.63	-0.64	0.53	0.49	-0.72	-0.07	0.53	-0.65	-0.30	0.10	0.17	-0.13	0.19	0.05	0.73	0.28	0.39	0.71	0.05	0.04	0.21	-0.66	0.50	0.41	-0.22	0.72	0.51	0.11	-0.36	-0.66	-0.11	
Bifidobacteriaceae	0.05	0.72	0.00	0.70	-0.54	-0.68	0.72	-0.46	1.00	-0.77	-0.32	0.55	0.13	0.62	-0.57	-0.52	0.50	0.13	0.23	0.47	-0.05	0.08	0.03	0.52	0.15	0.47	-0.77	-0.48	-0.58	<b>0.90</b>	0.13	0.09	0.64	<b>0.58</b>	-0.88	-0.28	0.11	-0.18	-0.58	0.15	-0.14	0.50	0.37
Bradyrhizobiaceae	-0.21	-0.65	0.16	0.63	0.40	0.75	<b>-0.85</b>	0.65	-0.77	1.00	0.47	-0.52	-0.10	-0.62	0.45	0.48	-0.63	0.07	-0.22	-0.65	-0.10	0.05	0.33	-0.20	-0.50	<b>0.99</b>	0.53	0.43	<b>-0.87</b>	-0.08	0.44	0.55	-0.37	0.80	0.27	0.11	0.23	0.80	-0.38	-0.08	-0.62	-0.30	
Cellulomonadaceae	-0.73	-0.30	<b>0.83</b>	-0.10	0.07	0.68	-0.23	0.00	-0.32	0.47	1.00	0.35	0.12	0.10	-0.37	0.02	0.27	0.67	0.20	0.05	0.60	-0.43	0.73	0.05	-0.80	-0.75	0.25	0.42	0.17	-0.22	-0.23	0.61	0.07	0.38	0.13	-0.02	-0.66	-0.57	0.22	-0.20	0.41	0.12	-0.48
Clostridiaceae	-0.44	0.34	0.52	-0.80	-0.29	-0.02	0.57	-0.63	0.55	-0.52	0.35	1.00	0.12	<b>-0.92</b>	-0.98	0.75	<b>0.92</b>	0.38	0.55	<b>0.90</b>	0.38	-0.22	0.22	0.13	-0.45	0.02	-0.57	-0.03	-0.22	0.58	0.20	0.09	-0.63	<b>0.90</b>	-0.68	-0.28	-0.35	<b>-0.85</b>	-0.58	-0.23	0.13	<b>0.92</b>	0.23
Comamonadaceae	0.24	-0.10	-0.03	-0.35	-0.46	0.00	0.42	-0.63	0.13	-0.10	0.12	1.00	0.13	-0.08	-0.07	0.22	0.10	0.65	0.07	0.25	0.32	0.12	0.23	-0.28	-0.57	-0.28	-0.15	-0.50	0.33	-0.42	0.46	-0.04	0.25	0.06	-0.41	-0.18	-0.33	-0.21	-0.05	0.10	0.17	-0.25	
Coriobacteriaceae	-0.18	0.25	0.26	-0.78	-0.26	-0.12	0.72	-0.64	0.62	-0.62	0.10	<b>0.92</b>	0.13	<b>1.00</b>	<b>-0.87</b>	-0.63	<b>0.90</b>	0.15	0.63	<b>0.92</b>	0.23	-0.18	0.10	0.27	-0.15	0.22	-0.62	-0.23	-0.10	0.60	0.17	0.00	-0.66	<b>0.83</b>	-0.69	-0.16	-0.19	-0.72	-0.66	-0.18	-0.06	<b>0.97</b>	0.38
Cytophagaceae	0.48	-0.38	-0.57	0.77	0.28	0.03	-0.50	0.53	-0.57	0.45	-0.37	<b>-0.98</b>	-0.08	<b>-0.87</b>	<b>1.00</b>	<b>0.78</b>	<b>-0.85</b>	-0.42	-0.53	<b>-0.85</b>	-0.33	-0.30	-0.32	-0.10	0.48	0.00	0.50	-0.02	0.28	-0.55	-0.23	-0.15	-0.68	<b>-0.88</b>	0.66	0.31	0.88	0.80	0.54	0.27	0.20	<b>-0.87</b>	-0.22
Deinococcaceae	0.39	-0.35	-0.34	0.73	0.11	0.13	-0.37	0.49	-0.52	0.48	0.02	-0.75	-0.07	-0.63	0.78	<b>1.00</b>	-0.67	-0.32	-0.28	-0.78	-0.22	0.07	0.20	0.17	0.40	-0.20	0.40	-0.30	0.30	0.53	-0.67	0.13	0.44	-0.63	0.43	0.14	0.07	0.53	0.38	0.35	-0.05	-0.67	-0.58
Enterobacteriaceae	0.40	0.18	0.48	<b>-0.88</b>	-0.11	0.07	0.73	-0.72	0.50	0.63	0.27	<b>0.92</b>	0.22	<b>0.90</b>	<b>-0.85</b>	-0.67	<b>1.00</b>	0.43	0.55	<b>0.92</b>	0.60	0.45	0.13	0.32	-0.45	-0.02	-0.68	-0.03	-0.10	0.65	0.15	0.00	-0.54	0.77	-0.60	-0.08	-0.39	-0.77	-0.65	0.02	-0.19	<b>0.97</b>	0.23
Eubacteriaceae	<b>-0.89</b>	-0.10	<b>0.94</b>	-0.57	0.18	0.25	0.08	-0.07	0.13	0.07	0.67	0.38	0.10	0.15	-0.42	-0.32	0.43	1.00	-0.02	0.15	<b>0.81</b>	-0.78	<b>0.97</b>	0.43	<b>-0.83</b>	-0.47	-0.08	0.53	0.00	0.27	0.15	0.56	-0.03	0.37	-0.08	0.28	-0.68	-0.30	0.08	0.15	-0.68	0.12	-0.13
Fusobacteriaceae	-0.14	-0.10	0.08	-0.43	-0.24	0.00	0.60	-0.53	0.23	-0.22	0.20	0.55	0.65	0.63	-0.53	-0.28	0.55	-0.02	1.00	0.55	0.17	-0.27	0.02	-0.17	-0.25	-0.37	-0.33	-0.25	-0.32	0.25	-0.33	0.29	-0.61	0.48	-0.19	-0.44	-0.18	-0.57	-0.61	-0.17	0.10	-0.70	-0.12
Flavobacteriaceae	0.24	0.23	0.25	-0.75	-0.10	-0.12	0.65	-0.65	0.47	-0.65	0.05	<b>0.90</b>	0.07	<b>-0.92</b>	<b>-0.85</b>	-0.78	<b>0.92</b>	0.15	0.55	1.00	0.32	0.15	-0.08	0.02	0.27	0.20	-0.60	-0.03	-0.08	0.53	0.33	-0.26	-0.60	0.70	-0.57	-0.15	-0.33	-0.72	-0.69	-0.17	0.03	<b>0.97</b>	0.43
Gemmatimonadaceae	-0.69	-0.28	0.76	-0.57	0.27	0.25	0.30	-0.30	-0.05	0.10	0.60	0.38	0.25	0.23	-0.33	-0.22	0.60	<b>0.81</b>	0.17	0.32	1.00	<b>0.87</b>	0.60	0.42	-0.80	-0.55	-0.25	0.42	0.13	0.25	-0.03	0.28	0.00	0.25	0.00	0.28	0.69	-0.40	-0.15	0.33	-0.54	0.38	-0.22
Geodermatophilaceae	0.53	0.38	-0.65	0.53	-0.35	-0.08	-0.40	0.10	-0.08	0.05	-0.43	-0.22	-0.32	-0.18	0.20	0.07	-0.45	-0.78	-0.27	-0.15	<b>0.87</b>	1.00	0.58	-0.70	0.62	0.50	0.20	-0.28	-0.08	-0.25	0.13	0.48	0.15	-0.12	-0.03	-0.38	0.69	0.10	0.18	-0.47	0.78	-0.18	0.22
Hyphomicrobiaceae	-0.78	-0.06	<b>0.87</b>	-0.25	0.13	0.27	-0.17	0.17	-0.03	0.33	0.73	0.22	0.12	-0.10	-0.32	-0.20	0.13	<b>0.87</b>	-0.02	-0.08	0.60	-0.58	1.00	0.18	<b>-0.85</b>	-0.65	0.15	0.53	-0.20	0.02	-0.02	0.64	-0.08	0.20	0.08	0.00	-0.68	-0.20	0.17	0.05	-0.68	-0.08	-0.40
Iamiaceae	-0.10	0.03	0.27	-0.48	-0.08	-0.33	0.60	-0.13	0.52	-0.33	0.05	0.13	0.23	0.27	-0.10	0.17	0.32	0.43	0.17	0.02	0.42	0.70	1.00	-0.02	-0.03	-0.47	-0.35	-0.10	0.53	0.33	0.46	-0.28	0.18	-0.40	0.23	-0.32	-0.10	-0.30	0.60	-0.64	0.13	-0.15	
Kineosporiaceae	0.80	0.21	<b>-0.87</b>	0.43	-0.16	-0.45	0.02	0.19	0.15	-0.20	-0.80	-0.45	-0.28	-0.15	0.40	-0.45	<b>-0.83</b>	-0.25	-0.27	-0.80	-0.62	<b>-0.85</b>	-0.02	1.00	0.73	-0.03	-0.63	0.07	-0.05	-0.07	-0.45	0.04	-0.37	-0.13	0.01	0.66	0.55	-0.10	0.44	-0.23	0.27		
Lachnospiraceae	0.32	0.44	-0.49	-0.03	-0.05	0.50	0.15	0.05	0.47	-0.50	-0.75	0.02	-0.57	0.22	0.00	0.48	0.47	-0.37	0.20	0.20	0.62	0.50	-0.02	0.47	-0.37	1.00	-0.25	-0.															

Variables	Al <sub>2</sub> O <sub>3</sub>	As	Ba	CaO	Co	Cr	Cu	Fe <sub>2</sub> O <sub>3</sub>	K <sub>2</sub> O	MgO	MnO	Mo	Na <sub>2</sub> O	Ni	P <sub>2</sub> O <sub>5</sub>	Pb	Rb	SiO <sub>2</sub>	Sr	TiO <sub>2</sub>	U	V	Y	Zn	Zr
	<i>Acetobacteraceae</i>	-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
<i>Acidobacteriaceae</i>	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
<i>Bacillaceae</i>	-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
<i>Bacteroidaceae</i>	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
<i>Beijerinckiaceae</i>	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
<i>Bifidobacteriaceae</i>	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
<i>Bradyrhizobiaceae</i>	-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
<i>Cellulomonadaceae</i>	-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
<i>Clostridiaceae</i>	-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
<i>Comamonadaceae</i>	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
<i>Coriobacteriaceae</i>	-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
<i>Cytophagaceae</i>	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
<i>Deinococcaceae</i>	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
<i>Enterobacteriaceae</i>	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
<i>Eubacteriaceae</i>	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
<i>Flavobacteriaceae</i>	-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
<i>Fusobacteriaceae</i>	-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
<i>Gemmatimonadaceae</i>	0.62	-0.31	<b>0.85</b>	-0.14	-0.04	-0.13	0.02	-0.13	<b>0.85</b>	0.19	0.17	0.06	0.55	0.01	0.01	0.67	<b>0.87</b>	-0.18	0.48	0.27	0.45	-0.13	0.53	0.22	0.55
<i>Geodermatophilaceae</i>	-0.78	0.33	<b>-0.92</b>	0.10	-0.15	-0.17	-0.04	-0.07	<b>-0.98</b>	-0.35	-0.30	-0.20	-0.43	-0.27	-0.29	<b>-0.83</b>	<b>-0.97</b>	0.33	-0.56	-0.43	-0.66	-0.07	-0.68	-0.38	-0.73
<i>Hyphomicrobiaceae</i>	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
<i>Iamiaceae</i>	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	<b>0.90</b>	0.34	0.77	0.55	0.40
<i>Kineosporiaceae</i>	-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
<i>Lachnospiraceae</i>	-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
<i>Methylobacteriaceae</i>	-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
<i>Micrococcaceae</i>	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
<i>Micromonosporaceae</i>	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
<i>Moraxellaceae</i>	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
<i>Nocardioideae</i>	-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
<i>Oscillospiraceae</i>	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
<i>Oxalobacteraceae</i>	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
<i>Pseudomonadaceae</i>	-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
<i>Rhodobacteraceae</i>	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
<i>Rubrobacteraceae</i>	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
<i>Ruminococcaceae</i>	-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
<i>Solirubrobacteraceae</i>	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
<i>Sphingobacteriaceae</i>	-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
<i>Sphingomonadaceae</i>	0.73	-0.01	0.48	0.68	0.67	0.60	0.60	0.50	0.48	<b>0.93</b>	0.68	0.68	0.75	0.66	0.68	0.59	0.49	-0.82	<b>0.94</b>	<b>0.85</b>	0.61	0.62	0.70	<b>0.85</b>	0.33
<i>Staphylococcaceae</i>	-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
<i>Veillonellaceae</i>	-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
<i>Xanthomonadaceae</i>	-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 standardized ( $\log_{10}$ )

**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 ( $\alpha$ ,  $\gamma$ ,  $\delta$  and  $\epsilon$ ). 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*, *Nocardiosis* 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)  $\gamma$  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) halophily in *Alterococcus*, *Salinarimonas*, and *Nocardioidea* (*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 *Verrucosipora*) were also present. Other species linked to aquatic life, some of them motile including: (a)

Actinobacteria—*Cryptosporangium*, *Nocardioidea* 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  $\beta$ - and  $\alpha$ -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, Opiritaceae and Rhodospirillaceae were present. Facultative anaerobes including the Enterobacteriaceae, Myxococcaceae, some Opiritaceae 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 ( $\alpha$ -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  $\alpha$ -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 Geodermatophilaceae 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; Urzi *et al.*, 2001). Black pigmented Geodermatophilaceae show high stress resistances, including to massive doses of  $\gamma$  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. pluviialis* 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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## References

- Alfaro SC, Gaudichet A, Gomes L, Maille M. (1998). Mineral aerosol production by wind erosion: aerosol particle sizes and binding energies. *Geophys Res Lett* **25**: 991–994.
- Ardley JK, Parker MA, De Meyer SE, Trengove RD, O'Hara GW, Reeve WG et al. (2012). *Microvirga lupini* sp. nov. *Microvirga lotononidis* sp. nov. And *Microvirga zambiensis* sp. nov. are alphaproteobacterial root-nodule bacteria that specifically nodulate and fix nitrogen with geographically and taxonomically separate legume hosts. *Int J Syst Evol Microbiol* **62**: 2579–2588.
- Bristow GS, Hudson-Edwards KA, Chappell A. (2010). Fertilizing the Amazon and equatorial Atlantic with West African dust. *Geophys Res Lett* **37**: 3–7.
- Callot Y, Marticorena B, Bergametti G. (2000). Geomorphologic approach for modelling the surface features of arid environments in a model of dust emissions: application to the Sahara desert. *Geodinamica Acta* **13**: 246–270.
- Caquineau S, Gaudichet A, Gomes L, Magonthier M-C, Chatenet B. (1998). Saharan dust: clay ration as a relevant tracer to assess the origin of soil-derived aerosols. *Geophys Res Lett* **25**: 983–986.
- Chanal A, Chapon V, Benzerara K, Barakat M, Christen R, Achouak W et al. (2006). The desert of Tataouine: an extreme environment that hosts a wide diversity of microorganisms and radiotolerant bacteria. *Environ Microbiol* **8**: 514–525.
- Chappell A, Warren A, O'Donoghue A, Robinson A, Thomas A, Bristow C. (2008). The implications for dust emission modeling of spatial and vertical variations in horizontal dust flux and particle size in the Bodele Depression, Northern Chad. *J Geophys Res* **113**: D04214.
- Collaud Coen M, Weingartner E, Schaub D, Hueglin C, Corrigan C, Schwikowski M et al. (2003). Saharan dust events at the Jungfraujoch: detection by wavelength dependence of the single scattering albedo and analysis of the events during the years 2001 and 2002. *Atmos Chem Phys Discuss* **3**: 5547–5594.
- Darwin CR. (1846). An account of the fine dust which often falls on vessels in the Atlantic Ocean. *Q J Geol Soc London* **2** (Read 4 June 1845), 26–30. <http://darwinonline.org.uk/content/frameset?itemID=F1672&viewtype=side&pageseq=1>.
- Drake N, Bristow CS. (2006). Shorelines in the Sahara: Geomorphological evidence for an enhanced monsoon from palaeolake Megachad. *Holocene* **16**: 901–911.
- Dreyfus BL, Dommergues YR. (1981). Nodulation of *Acacia* species by fast- and slow-growing tropical strains of *Rhizobium*. *Appl Environ Microbiol* **41**: 97–99.
- Engelstaedter S, Tegen I, Washington R. (2006). North African dust emissions and transport. *Earth Sci Rev* **79**: 73–100.
- Eppard M, Krumbein WE, Koch C, Rhiel E, Staley JT, Stackebrandt E. (1996). Morphological, physiological, and molecular characterization of actinomycetes isolated from dry soil, rocks, and monument surfaces. *Archiv Microbiol* **166**: 12–22.
- Ettl H, Gärtner G. (1995). *Syllabus der Boden-, Luft- und Flechtenalgen*. Gustav Fischer Verlag: Stuttgart, 721pp.
- Friedl T. (1995). Inferring taxonomic positions and testing genus level assignments in coccoid green algae: a phylogenetic analysis of 18S ribosomal RNA sequences from *Dictyochloropsis reticulata* and from members of the genus *Myrmecia* (Chlorophyta, Trebouxiophyceae cl. nov.). *J Phycol* **31**: 632–639.
- Garrity GM, Heimbuch BK, Gagliardi M. (1996). Isolation of zoosporegenous actinomycetes from desert soils. *J Ind Microbiol* **17**: 260–267.
- Giles J. (2005). The dustiest place on Earth. *Nature* **434**: 816–819.
- Giongo A, Favet J, Lapanje A, Gano KA, Kennedy S, Brown C et al. (2012). Microbial hitchhikers on intercontinental dust: high-throughput sequencing to catalogue microbes in small sand samples. *Aerobiologia* (in press).
- Gorbushina AA, Kort R, Schulte A, Lazarus D, Schnetger B, Brumsack H-J et al. (2007). Life in Darwin's dust - intercontinental transport and survival of microbes in the nineteenth century. *Environ Microbiol* **9**: 2911–2922.
- Goudie AS, Middleton NJ. (2001). Saharan dust storms: nature and consequences. *Earth Sci Rev* **56**: 179–204.
- Goudie AS, Middleton NJ. (2006). *Desert Dust in the Global System*. Springer: Heidelberg, 288pp.
- Griffin DW. (2007). Atmospheric movement of microorganisms in clouds of desert dust and implications for human health. *Clin Microbiol Rev* **20**: 459–477.
- Griffin DW, Kellogg C, Shinn E, Gray M, Garrison G. (2003a). U.S. Geological Survey, St. Petersburg, Florida, Desert Storms and their ability to move microorganisms and toxins around the globe. <http://www.cprm.gov.br/pgagem/puerto/MedGeo.Griffin.pdf>.
- Griffin DW, Kellogg CA, Garrison VH, Lisle JT, Borden TC, Shinn EA. (2003b). African dust in the Caribbean atmosphere. *Aerobiologia* **19**: 143–157.
- Hirsch P, Gallikowski CA, Siebert J, Peissl K, Kroppenstedt R, Schumann P et al. (2004a). *Deinococcus frigens* sp nov, *Deinococcus saxicola* sp nov., and *Deinococcus marmoris* sp nov, low temperature and draught-tolerating, UV-resistant bacteria from continental Antarctica. *Syst Appl Microbiol* **27**: 636–645.
- Hirsch P, Mevs U, Kroppenstedt RM, Schumann P, Stackebrandt E. (2004b). Cryptoenclolithic actinomycetes from antarctic sandstone rock samples: *Micromonospora endolithica* sp nov and two isolates related to *Micromonospora coerulea* Jensen 1932. *Systematic & Appl. Microbiol* **27**: 166–174.
- Hoshina R, Iwataki M, Imamura N. (2010). *Chlorella variabilis* and *Micractinium reisseri* sp. nov. (Chlorellaceae, Trebouxiophyceae): Redescription of the endosymbiotic green algae of *Paramecium bursaria* (Peniculia, Oligohymenophorea) in the 120th year. *Phycol Res* **58**: 188–201.
- Hungate B, Danin A, Pellerin NB, Stemmler J, Kjellander P, Adams JB et al. (1987). Characterization of manganese-oxidizing (MnII-MnIV) bacteria from Negev desert rock varnish - implications in desert varnish formation. *Canadian J Microbiol* **33**: 939–943.
- John DM, Whitton BA, Brook AJ (Eds.) (2002). *The Freshwater Algal Flora of the British Isles. An Identification Guide To Freshwater and Terrestrial Algae*. Cambridge University Press: Cambridge, pp 714.
- Kellogg CA, Griffin DW. (2006). Aerobiology and the global transport of desert dust. *Trends Ecol Evol* **21**: 638–644.
- Kellogg CA, Griffin DW, Garrison VH, Peak KK, Royall N, Smith RR et al. (2004). Characterization of aerosolized bacteria and fungi from desert dust events in Mali, West Africa. *Aerobiologia* **20**: 99–110.

- Koren I, Kaufman YJ, Washington R, Todd MC, Rudich Y, Martins JV *et al.* (2006). The Bodele Depression: a single spot in the Sahara that provides most of the mineral dust to the Amazon forest. *Environ Res Lett* **1** (2006) 014005 (5pp).
- Kröpelin S, Verschuren D, Lézine AM, Eggermont H, Cocquyt C, Francus P *et al.* (2008). Climate-driven ecosystem succession in the Sahara: the past 6000 years. *Science* **320**: 765–768.
- Lim N, Munday CI, Allison GE, O’Loingsigh T, De Deckker P, Tapper NJ. (2011). Microbiological and meteorological analysis of two Australian dust storms in April 2009. *Sci. Total Environ* **412-413**: 223–231.
- Luo W, Proschold T, Bock C, Krienitz L. (2010). Generic concept in *Chlorella*-related coccoid green algae (Chlorophyta, Trebouxiophyceae). *Plant Biol* **12**: 545–553.
- Macia-Vicente JG, Rosso LC, Ciancio A, Jansson H-B, Lopez-Llorca LV. (2009). Colonisation of barley roots by endophytic *Fusarium equiseti* and *Pochonia chlamydosporia*: effects on plant growth and disease. *Ann Appl Biol* **155**: 391–401.
- Mainguet M. (1995). «*L’homme et la sécheresse*». Masson: Paris 335. pp Prescott (Prescott p. 623).
- Middleton JJ, Goudie AS. (2001). Saharan dust: sources and trajectories. *Trans Inst Br Geogr NS* **26**: 165–181.
- Prescott LM, Harley JP, Klein DA. (2003). *Microbiologie*, 2nd French edn. Editions De Boeck Université: Bruxelles, pp. 668–696 (Chapter 30).
- Prospero JM, Blades E, Mathison G, Naidu R. (2005). Interhemispheric transport of viable fungi and bacteria from Africa to the Caribbean with soil dust. *Aerobiologia* **21**: 1–19.
- Rainey FA, Ray K, Ferreira M, Gatz BZ, Nobre F, Bagaley D *et al.* (2005). Extensive diversity of ionizing-radiation-resistant bacteria recovered from Sonoran desert soil and description of nine new species of the genus *Deinococcus* obtained from a single soil sample. *Appl Environ Microbiol* **71**: 7630–7630.
- Rypien KL. (2008). African dust is an unlikely source of *Aspergillus sydowii*, the causative agent of sea fan disease. *Mar Ecol-Progr Ser* **367**: 125–131.
- Shinn EA, Smith GW, Prospero JM, Betzer P, Hayes ML, Garrison VH *et al.* (2000). African dust and the demise of Caribbean coral reefs. *Geol Res Lett* **27**: 3029–3032.
- Shao Y-P. (2008). *Physics and Modelling of Wind Erosion* Springer Science + Business Media B.V, 452pp.
- Shao Y-P, Wyrwoll K-H, Chappell A, Huang J-P, Lin Z-H, McTainsh G *et al.* (2011). Dust cycle: an emerging core theme in earth system science. *Aeolian Res* **2**: 181–204.
- Staley JT, Palmer F, Adams JB. (1982). Microcolonial fungi: common inhabitants on desert rocks? *Science* **215**: 1093–1095.
- Toepfer I, Favet J, Schulte A, Schmölling M, Butte W, Triplett EW *et al.* (2012). Pathogens as potential hitchhikers on intercontinental dust. *Aerobiologia* **28**: 221–231.
- Urzi C, Brusetti L, Salamone P, Sorlini C, Stackebrandt E, Daffonchio D. (2001). Biodiversity of Geodermatophilaceae isolated from altered stones and monuments in the Mediterranean basin. *Environ Microbiol* **3**: 471–479.
- Urzi C, Realini M. (1998). Colour changes of Noto’s calcareous sandstone as related to its colonisation by microorganisms. *Int Biodeter Biodegr* **42**: 45–54.
- Vidhyavathi R, Venkatachalam L, Sarada R, Ravishankar GA. (2008). Regulation of carotenoid biosynthetic genes expression and carotenoid accumulation in the green alga *Haematococcus pluvialis* under nutrient stress conditions. *J Exp Bot* **59**: 1409–1418.
- Warren A, Chappell A, Todd MC, Bristow C, Drake N, Engelstaedter S *et al.* (2007). Dust-raising in the dustiest place on earth. *Geomorphology* **92**: 25–37.
- Weir-Brush JR, Garrison VH, Smith GW, Shinn EA. (2004). The relationship between gorgonian coral (Cnidaria: Gorgonacea) diseases and African dust storms. *Aerobiologia* **20**: 119–126.
- Yamaguchi N, Ichijo T, Sakotani A, Baba T, Nasu M. (2012). Global dispersion of bacterial cells on Asian dust. *Sci Rep* **2**: 0525.

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