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Identification of airborne bacteria of Saharan dust storm, Kuwait

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Abstract

Bacteria carried by desert dust storms have been involved in human disease. A major Saharan and Arabian Peninsula dust storm struck Kuwait on March 4, 2010, carrying large amounts of desert dust through heavily populated areas. Bacterial cultures obtained from dust storm samples were subjected to fatty acid profiling and 16S rDNA sequencing for species identification. Active bacteria isolated and identified included *Kokuria* spp, *Bacillus atrophaeus*, *Bacillus cereus*, *Micrococcus luteus*, *Arthrobacter agilis*, *Brevibacterium linens*, and *Curtobacterium flaccumfaciens*. Fatty acid analysis revealed a significant difference in bacterial community profile between the airborne samples of the dust storm and the calm periods. Bacteria carried by dust storms include plant and human pathogens. Thus, dust storms facilitate the dispersal of biological particles that may substantially impact downwind ecosystems and human health. The results indicate that there is strong potential for transport of human pathogens by major desert dust storms and there is a well-established link between airborne particulate inhalation and respiratory diseases. Nevertheless, many technological challenges still need to be overcome to fully understand the roles of airborne bacteria in our health and global ecosystems.

1. Introduction

The Arabian Peninsula is a region of frequent sandstorm activity due to its proximity to North African, Middle Eastern, and Central Asian deserts and its location upstream of the prevailing northeasterly winds (Washington, 2003). Kuwait lies approximately 3,500 km from the center of the Sahara desert in Niger and 1,000–2,500 km from Saharan regions of Egypt, Libya, Chad, and Sudan. The prevailing winds from the Sahara generally flow in northerly and northeastern directions, which can transport materials over the Mediterranean Sea, the Red Sea, and the Arabian Peninsula to Kuwait (Middleton and Goudie, 2001). In addition to the prevailing winds, strong pressure gradients or thunderstorm collapse outflows in the basin and Arabian Peninsula can produce hot dry winds with temperatures in excess of 54°C and humidity below 10%, an event known as a “Haboob”. A Haboob can carry dust thousands of miles within a wall of loose sand up to 100 km wide and 1–3 km in elevation (Chen and Fryrear, 2002).

These sandstorms carry dust swept up into an airborne cloud through saltation and ionization of small particles, a process that becomes more efficient in drier intense winds (Slobdlan, 1996). The primary desert soil is sand composed of silicon dioxide and

layered silicates with particle sizes that range from 0.6 to 1.0 mm in diameter, while the dust carried in a sandstorm typically consists of organic particles less than 0.1 mm together with lower-density clay and porous silicates between 0.002 and 0.1 mm. The Sahara Desert and adjacent zones transport approximately 190 kg of particle matter per hectare annually, with approximately 20% of the total particulate mass being organic material compared with less than 5% of the desert soil (Abdel-Hafez, 1982). Although dry and inert, desert soils are relatively rich in bacteria, containing up to 10⁹ bacteria per gram (Whitman *et al.*, 1998). Further, thousands of bacterial species are present in desert soil although the vast proportion of the bacterial load (~99.9%) is composed of dominant phyla from the genera *Proteobacteria*, *Acidobacteria*, *Arthrobacteria*, *Bacillus*, *Kuthia*, and *Actinobacteria* (Janssen, 2006).

A previous analysis of desert dust settled after a dust storm in Kuwait found 147 distinct bacterial colony forming units (CFUs) from 10 separate genera (Lyles *et al.*, 2005). The bacteria in these sandstorms have been linked to several diseases including pneumonia, termed Al Eskan disease, and desert dust pneumonitis (Korenyi-Both and Juncer, 1997). In addition to the potential human health impacts, the transport of large quantities of bacteria in mass

airborne migrations through sandstorms can influence local ecosystems, including water sources, plants, and animal populations (Martiny *et al.*, 2006).

Dust loads transported into the atmosphere from Saharan have been estimated at 500 million to 1 billion tons annually (Moulin *et al.*, 1998). Currently, many efforts are directed at reducing ambient concentrations of particulate materials produced by human activity. However, the biological burden presented by sandstorms has the potential of spreading a wide variety of microorganisms (fungi, virus, and bacteria) and their pathogenic products (spores, mycotoxins, endotoxins) to plants, marine environments (Shinn *et al.*, 2000), and humans (Griffin *et al.*, 2001).

There are factors influencing the composition of air borne bacterial Communities. Of these factors, seasonality, meteorological conditions, anthropogenic influences, and variability in bacterial sources play an influential role in shaping the abundance and composition of airborne bacterial communities across time and space (Smets *et al.*, 2016). How and to what extent these factors affect the bacterial communities is, however, very context dependent. This implies that airborne bacteria are no more than a collection of organisms dispersed from different sources, and therefore less likely to be part of an atmospheric ecosystem.

However, airborne bacterial communities are distinctly different from their source environments, possibly because many of the bacteria do not survive in the atmospheric environment (Bowers *et al.*, 2011). The specific selection pressure in the atmosphere may, additionally, be caused by other ecological factors, such as availability of certain substrate. This ecological point of view rather fits into the “atmosphere biome” hypothesis of Morris *et al.* (2011), whereby the airborne microbes actively interact with each other and with the environment.

Previous studies have utilized a broad range of technical approaches to identify the microorganisms transported by sand dust (SD). These methods include the culturing of viable specimens, microscopic analyses, and a combination of DNA extraction, polymerase chain reaction (PCR) amplification, and sequencing (culturing-PCR sequencing method: CPS). Fungi, bacteria, and viruses pathogenic to humans have been isolated and identified in cultures by CPS (Griffin *et al.*, 2001 and Griffin *et al.*, 2003). However, it is speculated that the species present in SD identifiable by currently available methods account for less than 1% of the total (Griffin *et al.*, 2001).

The estimated 4.5 million people of Kuwait may be at particularly high risk of aerosolized bacteria exposure from dust storms due to their proximity to the Sahara Desert and surrounding Arabian Peninsula deserts. The occurrence and intensity of the events has been confirmed by various satellite images, including those acquired by the Terra satellite (NASA). The purpose of the present study was to identify the

bacteria carried by a major dust storm over Kuwait on March 4, 2010. A study of the microbial distribution is vital to assess the potential health and ecological impacts from airborne bacteria in Kuwait.

2. Materials and Methods

2.1 Sampling and bacterial culture

Dust samples were collected by gravity deposition using collection plates of R2A agar in dipotassium phosphate buffer, a material shown to have a higher recovery rate for airborne bacteria than typical tryptic soy agar (Horgan *et al.*, 1999). Plates were exposed to air and shielded from direct sunlight for 6 h on monitoring days and then incubated at 25°C, 37°C, or 45°C. Two sets of samples at each location were collected during a major sandstorm occurring on March 4, 2010 and then again on clear days following at least 2 weeks of calm weather. Plates were covered after the 6 h sampling period and transported for analysis. The locations and dates of samples are shown in Table 1.

Table 1. Locations, sample numbers, and dates of sampling

Sample ID	District	Suburb	# of Samples	Date
HAW-1	Hawali	Al-Jabriya	5	March 4, 2010
HAW-2	Hawali	Al-Jabriya	5	April 15, 2010
ALJ-1	Al-Jahra	Al-Jahra	5	March 4, 2010
ALJ-2	Al-Jahra	Al-Jahra	5	April 21, 2010
ALF-1	Al-Farwaniya	Al-Rabia	5	March 4, 2010
ALF-2	Al-Farwaniya	Al-Rabia	5	June 15, 2010
ALA-1	Al-Ahamadi	Fahad Al-Ahmad	5	March 4, 2010
ALA-2	Al-Ahamadi	Fahad Al-Ahmad	5	July 18, 2010
ALS-1	Al-Asimah	Kuwait City	5	March 4, 2010
ALS-2	Al-Asimah	Kuwait City	5	August 2, 2010

Sites of dust sample collection were within populated regions of Kuwait (Fig. 1). During the central event on March 4, 2010, dust was swept up from North Africa, Egypt, Jordan, and Syria by late spring instability. The resulting plume carried by dust east-northeast, obscuring satellite views of Kuwait and the northwestern tip of the Arabian Gulf (Fig. 2).

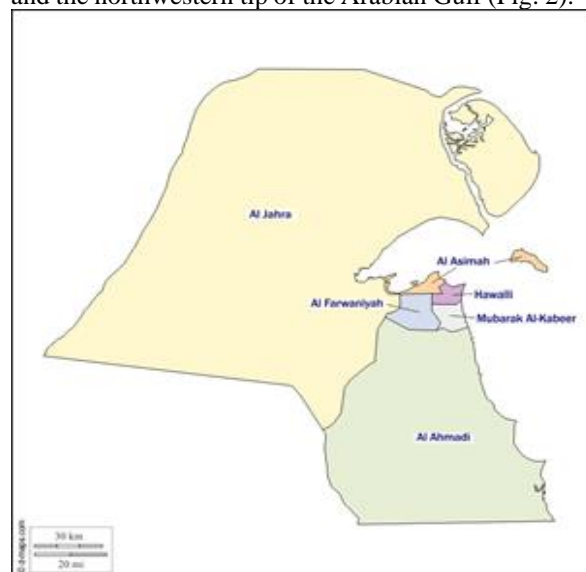


Figure 1. Locations of the sample’s districts, Kuwait.

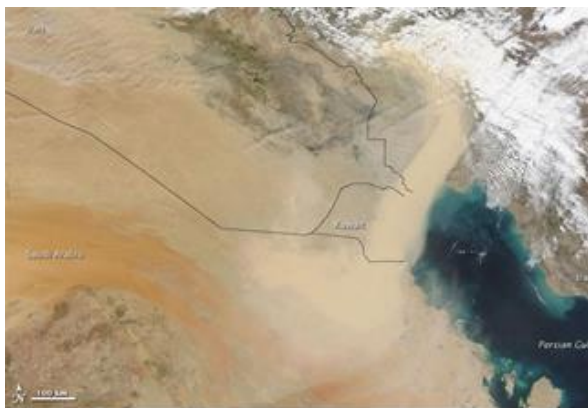


Figure 2. Satellite image obtained on March 4, 2010. Moderate true-color image captured on NASA's Terra satellite of the northwestern tip of the Arabian Gulf. The opaque dust plume beneath the arrow shows the location of the major event and the direction of travel from Kuwait into Iran.

2.2 Sample identification

Isolated bacterial samples were identified by the United Kingdom Food and Environment Research Agency. The samples were tested using a combination of fatty acid profiling (FAP) and partial sequencing of the 16S rRNA gene. The FAP profiles were compared with the commercially available TSBA6 6.10 Library supplied with MIDI Inc.'s Sherlock FAP system, while the 16S sequences were searched against the basic local alignment search tool (BLAST; NIH). Combined FAP and 16S sequencing usually provides a strong indication of species identity.

2.3 Whole-Cell Fatty Acid Analysis

The bacterial colonies from sampling plates were seeded on tryptic soy agar and incubated at 28°C for 48 h. Approximately, 30-mg fresh weight of cells was harvested from each individual sample. Following lipid extraction, an aliquot of the phospholipid fraction equivalent to 25-mg dry matter was used for fatty acid analysis according to procedures outlined for atmospheric and geological analyses of microbes (Fang *et al.*, 2000). Extraction, separation, hydrolysis, and derivatization yielded four ester-linked (EL) and two nonester-linked (NEL) PLFA fractions, which were further separated and identified as individual PLFA compounds by gas chromatography–mass spectrometry (GC–MS). Briefly, fatty acid fractions were analyzed by a mass-selective detector (Hewlett-Packard 5971A, USA) and 5890 series II gas chromatography system (Hewlett-Packard) equipped with a HP-5 capillary column (Agilent; 50 m length, 0.2 mm internal diameter; coated with a 0.3- μ m-thick cross-linked 5% phenylmethyl rubber phase) using He as the carrier gas. Individual PLFA components were identified and quantified using the MIDI Aerobic Bacteria Library TSBA6, MIDI Anaerobic Bacteria Library Moore6, NCPPB library.

2.4 Statistical analysis

All PLFA analyses were performed in duplicate. Concentrations of all individual PLFAs (log₁₀ mol%) were subjected to principal component analysis using SPSS 10.0 for Windows (IBM, USA) to elucidate major variation patterns. Components were assigned a similarity index (SI), and those with high similarity to known profiles were subjected to further 16S rRNA sequencing. Differences in the mean proportions of various PLFAs between sampling sites and times (during and after the dust storm event) were tested by Student's sample t-test.

2.5 16S rDNA Typing

The 16S rDNA was amplified using 8f-(5'-AGAGTTTGATCCTGGCTCAG-3') and 1492r (5'-TACGGATACCTTGTAGCACTT-3') primers in a 50- μ l PCR reaction mixture containing 200 μ M dNTPs, 50 μ M of each primer, 1 \times PCR buffer, 3 U Taq DNA polymerase, and 100 ng genomic DNA. The thermocycle consisted of an initial denaturation at 94°C for 4 min, followed by 35 cycles of 94°C for 1 min, 52°C for 1 min, and 72°C for 2 min, followed by a final extension at 72°C for 8 min. The 16S rDNA was gel purified, ligated to a PCR-TRAP cloning vector, and transformed into *Escherichia coli*. Individual 16S rDNA sequences were compared using BLAST (NIH).

3. Results

One Bacterial colonies isolated and amplified from the sample plates, during March 4, 2010; dust storm and calm periods thereafter, were first analyzed for fatty acid profiles by gas chromatograph. Similarity analyses of these profiles against libraries of GC–MS data revealed the presence of potential human, plant, and animal pathogens (Table 2).

Isolates included six bacterial genera: *Kokuria*, *Bacillus*, *Micrococcus*, *Arthrobacter*, *Brevibacterium*, and *Curtobacterium*. The calculated Sis, for bacteria collected at the different sampling sites on different days and the tentative taxonomic identifications, are shown in Table (2). The correlations between sites are less apparent from the table as the identification of likely bacterial genera was determined from entire libraries and some bacterial strains share fatty acid residues.

A more precise species/strain determination can be made using PCR sequencing of 16S rRNA. Thus, all samples with high SIs by gas chromatography were subjected to partial sequencing of the 16S rRNA gene. The details of these analyses for each sample site are tabulated in Table (2).

HAW-1

Fatty acid analysis indicated that sample plates at HAW-1 yielded a Gram-positive microbe that closely matched *Kokuria* spp. with SI of 0.733. Ensuing 16S rRNA sequencing indicated that an organism is closely related to *Arthrobacter* spp. Both organisms are in the family Micrococcaceae.

HAW-2

Fatty acid analysis indicated that the microbe from sample HAW-2 was likely *B. cereus* (SI = 0.645) with no other close matches. Sequencing of 16S rRNA also indicated *B. cereus*.

ALJ-1

Fatty acid analysis indicated that this colony was likely *M. luteus*. The relatively low SI of 0.548 normally indicates that the organism is not very closely related to any taxon in the library. However, 16S rRNA sequencing indicated that an organism is closely related to *Kokuria* spp, and both these organisms are in the family Micrococcaceae.

ALJ-2

The closest match by fatty acid analysis was *B. atropheus* (SI = 0.849), and 16S rRNA sequencing also indicated *Bacillus* spp. with similarities to *B. atropheus* and *Bacillus subtilis*.

ALF-1

The closest match by fatty acid analysis was *A. agilis* (SI = 0.753), while 16S rRNA sequencing indicated that an organism is closely related to *Kokuria* spp. As with 5 NA, both these organisms are in the family Micrococcaceae.

ALF-2

The closest match by fatty acid analysis was *Kurthia* but with a relatively low SI of 0.422. Subsequent 16S rRNA sequencing indicated that an organism is closely related to *Planococcus*. Both organisms are in the family Planococcaceae.

ALA-1

The closest match by fatty acid analysis was *Arthrobacter globiformis* (SI = 0.491), and 16S rRNA sequencing also indicated that a species is closely related to *Athrobacter* spp.

ALA-2

The closest matches by fatty acid analysis were *Curtobacterium* spp. (top match *C. flaccumfaciens* subsp. oortii or betae, SI = 0.658) and *Brevibacterium* spp. (top match *B. linens*, SI = 0.693). Sequencing of 16S rRNA indicated that the colony was likely *Brevibacterium* spp. Both *Curtobacterium* spp. and *Brevibacterium* spp are in the suborder Micrococcineae.

ALS-1

The closest match by fatty acid analysis was *B. cereus* (SI 0.645) with no other close matches. Similarly, 16S rRNA sequencing also indicated *B. cereus*.

ALS-2

The closest match was *Kurthia* but with a relatively low SI of 0.361. Sequencing of 16S rRNA indicated an organism closely related to *Planococcus*. Both *Kurthia* and *Planococcus* belong to the family Planococcaceae.

Figure (3) shows the structural fatty acid similarities of the samples collected during the study. Correlations looked at the relative abundance of fatty acid identification from retention time and relative peak height and determined the similarities between samples. Since fatty acid analysis represents all

living expressed parts of a community the exact comparison of one sample of bacteria to another is not definitive as some bacteria express similar fatty acids. There were considerable differences noted among populations. In particular, the groupings were separated by the differentiation at the 20% similarity with the presence of the March 4 sandstorm. The secondary groupings do not show a relative pattern based on geography, however, the Fahadal samples taken in the southernmost location do not show a similarity grouping with the remaining non-sandstorm samples at even the 20% similarity coefficient.

Table 2. Bacterial strains determined by analysis of fatty acids and correlated to a SI with known library scans.

Sample ID	Taxonomic affiliation	Sim index	Library	Date
HAW-1	<i>Kokuria rhizophila</i>	0.733	TSBA6	March 4, 2010
	<i>Bacillus luciferensis</i>	0.550		
	<i>Micrococcus luteus</i> GC sub B	0.531		
	<i>Micrococcus lylae</i> GC sub A	0.491		
HAW-2	<i>Bacillus cereus</i>	0.645	TSBA6	April 15, 2010
ALJ-1	<i>M. luteus</i> C subC	0.548	TSBA6	March 4, 2010
	<i>Bacillus megaterium</i> GC SubA	0.467		
	<i>M. lylae</i> GC sub	0.419		
	<i>B. luciferensis</i>	0.378		
ALJ-2	<i>Bacillus atropheus</i>	0.849	TSBA6	April 21, 2010
	<i>Viribacillus pantothenticus</i>	0.536		
	<i>Clavibacter michiganensis</i>	0.012		
ALF-1	<i>Arthrobacter agilis</i>	0.753	TSBA6	March 4, 2010
	<i>Brevibacillus choshinesis</i>	0.647		
	<i>Kocuria rosea</i> GC SubA	0.578		
	<i>Paenibacillus polymyxa</i>	0.495		
	<i>Athrobacter oxydans</i>	0.487		
	<i>Arthrobacter globiformis</i> GC sub	0.479		
	<i>Arthrobacter aurescens</i>	0.460		
ALF-2	<i>Curtobacterium albidum</i>	0.026	NCPBP3	
	<i>K. kurthia</i>	0.422		
	<i>Pseudomonas</i>	0.121		
ALA-1	<i>A. globiformis</i>	0.491	TSBA6	March 4, 2010
	<i>A. oxydans</i>	0.318		
	<i>C. albidum</i>	0.214		
ALA-2	<i>Crutobacterium flaccumfaciens betae</i>	0.658	TSBA6	July 18, 2010
	<i>Brevibacterium linens</i>	0.639		
	<i>Curtobacterium luteum</i>	0.569		
	<i>Brevibacterium epidermis</i>	0.516		
	<i>Brevibacterium casei</i>	0.427		
	<i>Curtobacterium albidum</i>	0.347		
	<i>Curtobacterium citreum</i>	0.239		
	<i>Clavibacter michiganensis</i>	0.192		
ALS-1	<i>B. cereus</i>	0.418	TSBA6	March 4, 2010
	<i>B. luciferensis</i>	0.387		
	<i>Pseudomonas</i>	0.212		
ALS-2	<i>Kurthia kurthia</i>	0.361	TSBA6	August 2, 2010
	<i>Staphylococcus (undetermined)</i>	0.134		

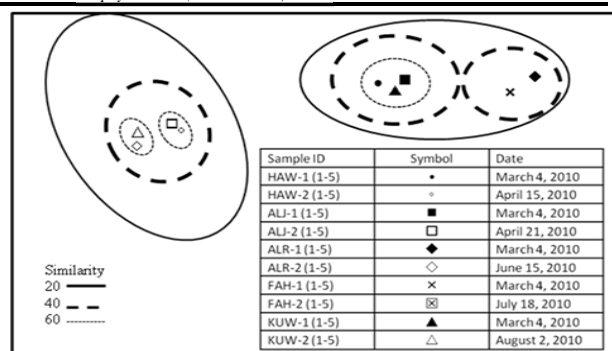


Figure 3: Correlation groupings showing similarity group linkages between samples collected at different dates and time points for fatty acid composition. 2Dimensional plots were produced from the resulting proximity matrices.

4. Discussion and conclusion

Fatty acid analysis revealed a significant difference in bacterial community profile between the airborne samples collected on March 4, 2010 dust storm and those collected during subsequent calm periods. Indeed, there was less than 20% similarity at the same location following the dust storm, while the level of similarity among individual samples taken on the day of the dust storm was greater than 40%, with individual clusters inside the communities showing greater than 60% similarity. As fatty acid analysis represents all the microbes in a community and provides reasonable validity for assignment of specific genotypes, these results indicate that dust storms markedly alter the local bacterial profile by depositing airborne species from other regions.

Taxonomic identification by 16S rRNA sequencing matched that by fatty acid analysis reasonably well for most samples, indicating at least a familial relationship. These analyses identified seven bacterial species with reasonable certainty, *Kokuria* spp, *B. atrophaeus*, *B. cereus*, *M. luteus*, *A. agilis*, *B. linens*, and *C. flaccumfaciens*, some of which may impact human health.

There is a well-established link between airborne particulate inhalation and respiratory diseases. Desert dust research in Iraq identified 149 bacteria CFUs with representatives from 10 genera, including *Mycobacterium*, *Brucella*, *Coxiella burnetii*, *Clostridium perferingens*, and *Bacillus*. Further, seven genera were isolated from the atmosphere over Erdemli, Turkey, during a Saharan dust event in March 2002 (Griffin *et al.*, 2002). *Kokuria* species, members of the Micrococcaceae family, are ubiquitous in nature and frequently found as normal skin flora in humans and other mammals. While documented infections due to *Kokuria* spp. are limited, *K. rosea* has been reported to cause catheter-related bacteremia (Altuntas *et al.*, 2004). *Bacillus* species are present in soil. *B. atrophaeus* is a Gram-positive, aerobic, endospore-forming, rod-shaped bacterium virtually identical to *B. subtilis* except that only the latter can form pigment in media containing an organic nitrogen source (Nakamura, 1989). Many of the isolates belonging to this species were previously classified as *B. subtilis* var. *niger* or even earlier as *Bacillus globigii*. Several of these strains are used in industry as indicators of sterilization (as pigmentation distinguishes them from background species) and as sources of restriction endonucleases. However, *B. cereus* tentatively identified at two sites is responsible for 2%–5% of all foodborne illnesses. Survival of the bacterial endospores when food is improperly cooked (at temperatures $\leq 100^{\circ}\text{C}/212^{\circ}\text{F}$) can cause severe nausea, vomiting, and diarrhea. *M. luteus* is also ubiquitous in the environment and found on human skin, water, dust, and soil. Although generally thought of as harmless, there have been rare cases of *Micrococcus* infections in people with compromised immune systems, as occurs in

HIV/AIDS. *Arthrobacter* is basic soil bacteria. All species in this genus are Gram-positive obligate aerobes that are rods during exponential growth and cocci in the stationary phase. *Brevibacterium* is a genus of the order Actinomycetales and the sole genus in the family *Brevibacteriaceae*. *B. linens* is ubiquitously present on the human skin, where it causes foot odor. The same bacterium is also employed to ferment several cheeses such as Munster, Limburger, Port-du-Salut, Raclette, and Näsäl. Its aroma also attracts mosquitoes (Bernard, 1996). *C. flaccumfaciens* is a Gram-positive soil bacterium that causes disease in a variety of plants.

Positive identification of bacterial species in this study was limited as we focused on the identification and grouping of families. Nonetheless, the evaluation shows a greater diversity of airborne microorganisms during dust storms than during quiescent periods. Monitoring of airborne bacteria transported by dust storms is an important public health concern in Kuwait as these storms have the potential to distribute a variety of non-native pathogenic bacteria over areas with human populations.

We conclude that many native and non-native microorganisms can be aerosolized and transported great distances by airborne dust. Bacteria carried by dust storms include plant and human pathogens. Thus, dust storms facilitate the dispersal of biological particles that may substantially impact downwind ecosystems and human health.

Conflict of interest: The author declares no conflict of interest.

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