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Diversity and distribution of fungal communities within the hot springs of soda lakes in the Kenyan rift valley

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Fungi are highly diverse and versatile, with members growing under different environmental conditions including extreme environments. Although fungal communities in some extreme environments have been investigated in recent years, little is known about their structure and richness within the hot springs of soda lakes in Kenya. The aim of the study was to determine the biogeography and diversity of fungi from the hot springs of four Soda lakes. Water, sediment and microbial mat samples were collected in triplicates from Lakes Bogoria, Magadi, Elmenteita and Little Magadi in Kenya. 454-Pyrosequencing was used to sequence amplicons of Internal Transcribed Spacer (ITS) gene region of the total community DNA in order to explore the fungal community composition in twenty four samples collected. Sequences were analyzed using QIIME pipeline Version 1.8.0, while hierarchical clustering, non-metric dimensional scaling (NMDS) and diversity indices were carried out using the R programming language and the Vegan package. A total of 139,023 quality sequence reads were obtained from which, 2,179 operational taxonomic units (OTUs) were realized at 3% genetic distance. Three known phyla (*Ascomycota* [83.3%], *Basidiomycota* [15.8%], *Glomeromycota* [0.02%]) were identified. Richness, abundance and taxonomic analyses identified *Agaricomycetes* as the most abundant and diverse class within Basidiomycota. Sequences matching with *Ascomycota* had high affinities with seven known classes, with *Sordariomycetes* and *Dothideomycetes* being the most abundant and diverse classes. The most abundant OTUs showed the highest sequence similarity to *Cladosporium* sp., *Cladosporium cladosporioides, Pleosporales* sp., *Aureobasidium pullulans* and *Aspergillus oryzae.*

Key words: Hot springs, fungi, 454 pyrosequencing, diversity, extreme environments.

INTRODUCTION

Extreme environments like the hot springs, saline and/or alkaline lakes, deserts and the ocean beds are found in nature. They are believed to have harsh conditions unfit for normal life to exist (Satyanarayana et al., 2005).

Hypersaline environments are found in all continents such as Great Salt Lake, Utah, the alkaline soda lakes of Egypt (Wadi El-Natrun), the Dead Sea, the soda lakes of Antarctica, Big Soda Lake and Mono Lake in California (Cantrell et al., 2006; Grant and Sorokin, 2011). In Kenya, the soda lakes (Bogoria, Magadi and Elmenteita) found in the East African Rift Valley represent the major type of naturally occurring highly alkaline environments.

Soda lakes are alkaline with pH values often ranging between 9 and 12. They are characterized by high concentrations of carbonate salts, especially sodium carbonate and related salt complexes. Many soda lakes also contain high concentrations of sodium chloride and other dissolved salts, making them saline or hypersaline lakes (Gunde-Cimerman et al., 2000; Litchfield and Gillevet, 2002). These hypersaline and highly alkaline soda lakes are considered some of the most extreme aquatic environments on earth. Hot springs are scattered all over the globe. They are produced by geo-thermally heated groundwater (Kauze et al., 2006) with extreme temperatures ranging about 45°C and above (Bhavesh et al., 2004). Temperature is one of the most important factor controlling the activity and evolution of microorganisms.

Microbial communities can be found in the most diverse conditions of temperature, pressure, salinity and pH (Kumar et al., 2010), as they are not limited to specific environments. Fungi have a worldwide distribution, and grow in a wide range of habitats, including extreme environments such as deserts or areas with high salt concentrations (Vaupotic et al., 2008) or ionizing radiation (Dadachova et al., 2007), as well as in deep sea sediments. Fungi play vital roles in the ecosystem. They are essentially decomposers, symbionts and pathogens that live closely with bacteria, plants and animals. Despite their functional importance, diversity, distribution and ecology of fungi is much less studied compared to bacteria (Desprez-Loustau et al., 2007). Traditional culturing methods that rely on morphological and other phenotypic characteristics as the main criteria for fungal classification (Bartnicki-Garcia, 1987) are heavily biased towards fast-growing species. Many fungi have specialized growth requirements, so this approach recovers only a small proportion of the community sampled (O'Brien et al., 2005). Similarly, fruiting body collection is limited to the detection of species that frequently reproduce sexually unless long-term studies are conducted **(**Straatsma and Egli, 2001). Therefore, these traditional methods alone do not enable a reliable identification of fungi at lower taxonomic levels (Feau et al., 2009). Molecular taxonomy has partially solved this problem, allowing better classification of fungi species (Fávaro et al., 2011; Gehlot et al., 2012). Recent studies carried out using illumina sequencing revealed that the phyla Ascomycota and Basidiomycota were the dominant and diverse groups of fungi within the sediments and water samples collected from Lake Magadi and Little Magadi (Kambura et al., 2016). In order to comprehensively determine the fungal diversity within the hot springs of Kenyan soda lakes, the 454 amplicon pyrosequencing approach was used (Bates et al., 2011; Dumbrell et al., 2011), that is not selective and biased for specific microbial growth like the previously used traditional methods.

MATERIALS AND METHODS

Authority to conduct research

Permission to conduct research in Kenya was granted by the National Commission for Science, Technology and Innovation (NACOSTI). All other necessary documents to access and collect samples from the soda lakes were obtained from the National Environment and Management Authority (NEMA) and the Kenya Wildlife Services (KWS).

Study sites

This study was conducted on four hot springs of the Kenyan soda lakes. Lake Elmenteita is situated at 0º 27'S, 36° 15'E on the floor of the Kenyan Rift Valley at 1,776 m above sea level, some 20 km south-east of Nakuru town and has no direct outlet (Melack, 1988). The water temperatures in the lake range between 30 and 40°C, the alkalinity is high (pH above 9) with a high concentration of carbonates, chlorides and sulphates (Mwaura, 1999). Lake Bogoria is located at 0° 13′ 33″ N, 36° 05′ 41″ E and lies at altitude of 1,000 m above sea level. The lake waters are alkaline (pH 10.5) and saline (up to 100 g/L total dissolved salts). Around Lake Bogoria are some 200 hot springs with water temperatures ranging from 39 to 98.5°C. Nearly all these springs are very close to the lake or even inside the lake. Lake Magadi (saline and alkaline lake) is situated at 1° 52′S 36° 16′E and is approximately 100 km^2 in size, lying in a catchment of faulted volcanic rocks, north of Tanzania's Lake Natron. The hot springs with temperatures up to 86°C, discharge saline alkaline waters into the lake (Behr, 2002). Little Magadi (*Nasikie eng'ida*) is located at 1° 45' 00" S, 36° 17' 00" E and is about 40 km south of the Lake Magadi. Temperatures of the springs at Little Magadi measure between 81 and 83.6°C.

Measurement of physicochemical parameters

The geographical position of each site in terms of longitude, latitude and elevation was taken using Global Positioning System (GARMIN eTrex 20). During sampling, the temperature, electrical conductivity (EC), total dissolved solids (TDS) and dissolved oxygen (DO) of each sampling point were measured on site using Electrical Chemical Analyzer (Jenway - 3405), whereas the pH was measured with a portable pH-meter (Oakton pH 110, Eutech Instruments Pty. Ltd) and confirmed with indicator strips (Merck, range 5-10). Temperature was recorded at three distinctive

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Table 1. Summary of samples collected from the four soda lakes and their parameters.

Soda Lake	Latitude ºS	Longitude ºE	Elevation (m)	Sampling point	Sample label	Sample type	Temperature (°C)	pH	Dissolved oxygen (Mg/l)	EC (mS/cm)	TDS (mg/l)
Little Magadi	1° 43'08.9"	36° 16'15.8"	606	1	LM1	Mats	81.90	8.5	0.29	1	1
				3	LM3	Mats	76.30	8.5	0.01		
				2	LMb	Sediment	67.90	8.6	0.98		
				3	LMc	Sediment	76.30	8.5	0.01		
				1	LMd	Water	81.90	8.6	0.29		
Lake Magadi	2° 00′08.1 S	36° 13′53.0″	616	1	MM1	Mats	43.80	8.8	1.17		
				2	MM2	Mats	41.00	8.8	6.04		
				3	MM3	Mats	37.90	8.8	4.80		
				1	MMa	Sediment	43.80	8.8	1.17		
				2	MMb	Sediment	41.00	8.8	6.04		
				3	MMc	Sediment	37.90	8.8	4.80		
				1	MMd	Water	43.80	8.8	1.17		
Lake Bogoria	0°13′ 46.1″ N	36° 05′34.8″	1,000	1	B1	Mats	84.60	8.2	0.10		
				2	B2	Mats	77.70	8.3	0.23		
				3	B3	Mats	54.00	8.7	0.53		
				1	Ba	Sediment	84.60	8.2	0.10		
				2	Bb	Sediment	77.70	8.3	0.23		
				1	Bd	Water	84.60	9	0.10		
Lake Elmenteita	0º28'21.6″ S	36°15'27.9″	1,789	1	E1	Mats	45.00	8.7	2.67		
				2	E2	Mats	44.70	8.7	2.45		
				3	E3	Mats	33.80	8.8	3.12		
				1	Ea	Sediment	45.00	8.7	2.67		
				2	Eb	Sediment	44.70	8.7	2.45		
				3	Ec	Sediment	33.80	8.8	3.12		

points along the rivulets of each hot spring and assigned to all the sample types for that site.

Sample collection

Sampling was carried out on 8th to 11th July, 2014. Water samples were collected from the mouth of each hot spring (L. Magadi at 43.8ºC, pH 8.8, Little Magadi at 81.9°C, pH 8.6, L. Bogoria at 84.6ºC, pH 9.0 and L. Elmenteita at 45ºC, pH 8.7) in triplicates using one liter sterile containers. Wet sediments (500 g) and microbial mats (500 g) were collected from the floor of each rivulet in triplicates using sterile jam jars at three distinct points (rivulet point 1, 2 and 3) shown in Table 1 that differed in temperature (L. Magadi: 43.9, 41 and 37.9ºC; Little Magadi: 81.9, 76.3, and 67.9ºC; L. Bogoria 84.6, 77.7 and 54ºC, and L. Elmenteita: 45, 44.7 and 33.8ºC).

The samples were labeled properly and transported on dry ice in cool boxes to the laboratory at the Jomo Kenyatta University of Agriculture and Technology. Water for DNA extraction was filtered through a 0.22 µM Whatman filter paper using a water pump (model Sartorius 16824) and stored at -80°C. Pellets for DNA extraction were obtained from water samples by re-suspending the filter papers in phosphate buffer solution (pH 7.5), and centrifuging 5 mL of the suspension at 13000 rpm for

10 min.

DNA extraction

Total microbial DNA was extracted from 0.4 g of each sample of wet sediments and microbial mats in triplicates using phenol chloroform DNA extraction protocol as described by Sambrook et al. (1989). The microbial DNA extracted from triplicate samples were pooled during the precipitation stage, washed, air dried and stored at -20°C prior to PCR.

PCR amplification and 454 pyrosequencing

PCR amplification of the fungal ITS gene region of 18S rDNA from the microbial DNA was performed using ITS1 (5´TCCGTAGGTGAACCTTGCGG3´) and ITS4 (5´TCCTCCGCTTATTGATATGC3´) primers (White et al., 1990) with barcodes. Amplification proceeded in a 30 cycle PCR using the HotStarTaq Plus Master Mix Kit (Qiagen, USA) with initial heating at 94°C for 3 min, followed by 28 cycles of denaturation at 94°C for 30 s, annealing at 53°C for 40 s and extension at 72°C for 1 min, after which a final elongation step at 72°C for 5 min was performed. PCR products were purified using calibrated Ampure XP beads (Agencourt Bioscience Corporation, MA, USA) and visualized on 2% agarose gel to determine the success of amplification and the relative intensity of bands. Multiple samples were pooled together in equal proportions based on their DNA concentrations. The pooled and purified PCR products were used to prepare DNA library, according to the pyrosequencing protocol (Yu and Zhang, 2012). Sequencing was performed at Molecular Research DNA, MR DNA (www.mrdnalab.com, MR DNA Shallowater, TX, USA) utilizing the Roche 454 FLX titanium sequencing platform and reagents following the manufacturer's guidelines.

Processing of pyrosequencing data

Sequences were depleted of barcodes and primers using a proprietary analysis pipeline (www.mrdnalab.com, MR DNA, Shallowater, TX) developed at the service provider's laboratory. Low quality sequences were identified by denoising and filtered out of the dataset (Reeder and Knight, 2010). Sequences that were < 200 base pairs after phred20- based quality trimming, sequences with ambiguous base calls and those with homopolymer runs exceeding 6 bp were eliminated. Sequences were analyzed by a script optimized for high-throughput data to identify possible chimeras in the sequence files, and all definite chimeras were depleted (Gontcharova et al., 2010). *De novo* Operational Taxonomic Unit (OTU) clustering was done with standard UCLUST method using the default settings as implemented in QIIME pipeline Version 1.8.0 at 3% genetic distance (Caporaso et al., 2010). Taxonomy was assigned to each OTU using nucleotide Basic local Alignment Tool (BLASTn) against SILVA SSU Reference 119 database at default e-value threshold of 0.01 in QIIME (Quast et al., 2013).

The diversity within each sample (alpha diversity) was evaluated using the observed species metric (count of unique OTUs in each sample), richness (Chao1), Shannon (diversity) and evenness were calculated in QIIME. Comparisons between community dissimilarity and environmental conditions were carried out using environmentally fitted non metric dimensional scaling (NMDS) plots (Minchin, 1987) using the Vegan package in R (Oksanen et al., 2012).

Statistical analysis

Alpha diversity indices (Shannon, Simpson, richness, observed species and Evenness) in each sample were calculated using vegan package version 1.16-32 in R software version 3.1.3 (R Development Core Team, 2012; Zhao et al., 2014). Community and Environmental distances were compared using analysis of similarity (ANOSIM) test, based upon Bray-Curtis distance measurements with 999 permutations. Significance was determined at 95% confidence interval ($p = 0.05$). Non metric dimensional scaling (NMDS) and hierarchical clustering were carried out using the R programming language (DeLong et al., 2006) and the Vegan package (Oksanen et al., 2012). To support OTU-based analysis, taxonomic groups were derived from the number of reads assigned to each taxon at all ranks from domain to genus using the taxa_summary.txt output from QIIME pipeline Version 1.8.0.

RESULTS

Sampling

Microbial mats, sediments and water samples were randomly collected at three different locations in the hot springs of the soda lakes in Kenya. The metadata collected before sampling included the geographical position of each site in terms of latitude, longitude and elevation, temperature, pH, electrical conductivity, total dissolved solids and dissolved oxygen. The various samples collected from the four soda lakes and their parameters are summarized in Table 1.

Estimators for diversity and species richness of fungal communities

For the 24 sequenced samples, five from Little Magadi, seven from L. Magadi and six each from both L. Bogoria and L. Elmenteita (Table 1), exclusion of low-quality and short sequence reads yielded 139,023 fungal ITS reads. Out of these, L. Elmenteita recorded the highest reads (51,913) while little Magadi had the lowest reads (25,958). Lake Magadi recorded 34,718 reads and L. Bogoria had 26,434 reads. The wet sediments, sample Ea at 45°C from L. Elmenteita, had the highest reads (14,929). Among the microbial mats, sample MM2 at 41°C from L. Magadi recorded the highest reads (10,574) followed by sample B1 at 84.6°C from L. Bogoria that had 8,204 reads while sample LM1 at 81.9°C from Little Magadi had 7,678 reads. Richness (S) estimated the sediments at L. Magadi, sample MMb at 41°C, to be the richest site with 633 species. The evenness (J′) value was closer to 0 with the highest value (0.258) recorded in sample MM1 at 43.80°C which revealed less distribution in abundance among species. Simpson (1/D) and Shannon's index (H') indicated the sediments in sample MMc at 37.90°C to harbor the most diverse taxa with 11.66 and 3.739 values respectively (Table 2).

Comparison of fungal communities between different sampling sites

Using a 3% dissimilarity cut-off for clustering, the reads

Table 2. Diversity indices computed on all OTU-based fungal taxonomic units.

Sample	No. of sequence reads		No. of OTUs per site	Richness (S)	Shannon (H')	Simpson (I/D)	Evenness (J')
	Before quality check	After quality check					
LM1	8285	7678	295	114	2.23	4.76	0.082
LM3	5088	4733		97	1.794	4.12	0.062
LMb	3922	3534		21	0.2	1.06	0.058
LMc	6281	5792		21	1.025	2.28	0.133
LMd	4498	4221		74	1.486	3.02	0.06
MM1	6490	5924	394	50	2.558	9.47	0.258
MM2	11385	10575		154	2.108	3.92	0.053
MM3	3005	2904		159	1.587	1.98	0.031
MMa	3883	3598		262	1.658	2.1	0.02
MMb	194	184		633	2.629	2.79	0.022
MMc	7463	6926		404	3.739	11.66	0.104
MMd	4944	4607		37	1.407	2.13	0.11
B1	8709	8204	294	116	2.342	4.13	0.09
B2	7168	6729		63	1.787	4.21	0.095
B3	2295	2106		26	0.938	2.02	0.098
Ba	1695	1590		83	1.911	5.2	0.081
Bb	6487	6004		41	0.409	1.14	0.037
Bd	1936	1801		52	0.817	1.4	0.044
E1	7535	6868	1196	172	2.248	3.77	0.055
E2	8864	8165		31	0.795	1.44	0.071
E3	14251	13084		51	1.469	2.66	0.085
Ea	15874	14929		6	0.192	1.07	0.202
Eb	6862	6422		125	2.082	4.23	0.064
Ec	2601	2445		35	1.013	1.87	0.079

were grouped into different operational taxonomic units (OTUs). The Fungal OTUs common to the four sampling sites were then presented using a Venn diagram to compare the relationships between the four communities (Figure 1). The numbers of fungal OTUs obtained for each prefecture were as follows: The hot spring at L. Elmenteita recorded the highest OTUs (1,196) while the one at L. Bogoria had 294, the least OTUs in this study. Lake Magadi hot spring had 394 OTUs and that of little Magadi had 295 OTUs. The hot springs at L. Bogoria and Little Magadi shared 87 OTUs, L. Elmenteita and L. Magadi shared 82 OTUs, L. Bogoria and L. Elmenteita shared 71 OTUs and Little Magadi and L. Magadi shared 95 OTUs. All the four sites (hot springs) shared 31 OTUs (Figure 1).

Fungal community composition and structure analysis

All analyzed sequences were classified into three known fungal phyla namely *Ascomycota*, *Basidiomycota*, *Glomeromycota* and *unclassified fungi* phylum. *Ascomycota* represented the most dominant and diverse phyla while *Glomeromycota* was the least dominant (Figure 2).

Ascomycota in hot springs of soda lakes

The phylum *Ascomycota* had a relative abundance of 100% in samples B3 at 54°C, LMc at 67.9°C, MM1 at 43.9°C and MMd at 43.9°C, 99% in samples LM3 at 67.9°C, LMd at 81.9°C, MM2 at 41°C, MM3 at 37.9°C, B1 at 84.6°C, Bb at 77.7°C and Eb at 44.7°C. Sample Ec at 33.8°C had the lowest relative abundance of 29.9%. In addition, all samples had relative abundances above 50% apart from Ec at 33.8°C (29%), E3 at 33.8°C (28%) and MMb at 41°C (0%). Seven classes were identified namely; *Dothideomycetes*, *Eurotiomycetes*, *Saccharomycetes*, *Sordariomycetes*, Pezizomycetes, *Leotiomycetes* and *Lichinomycetes* (Figure 3). *Dothideomycetes* and *Eurotiomycetes* were the most dominant (91.7 and 70.8%, respectively) and diverse as

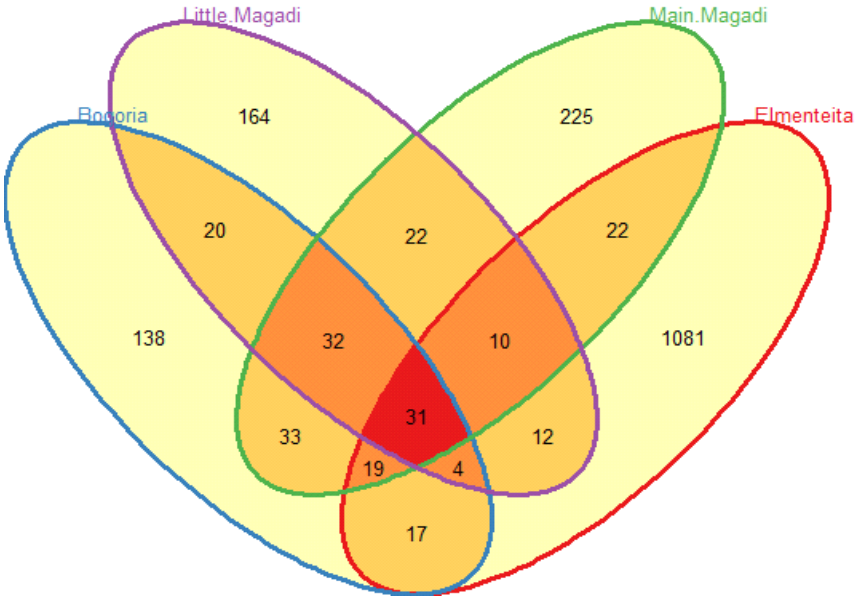

Figure 1. Venn diagram representing the number of fungal OTUs that are unique and shared between the samples from 4 different sampling sites. Main Magadi stands for Lake Magadi.

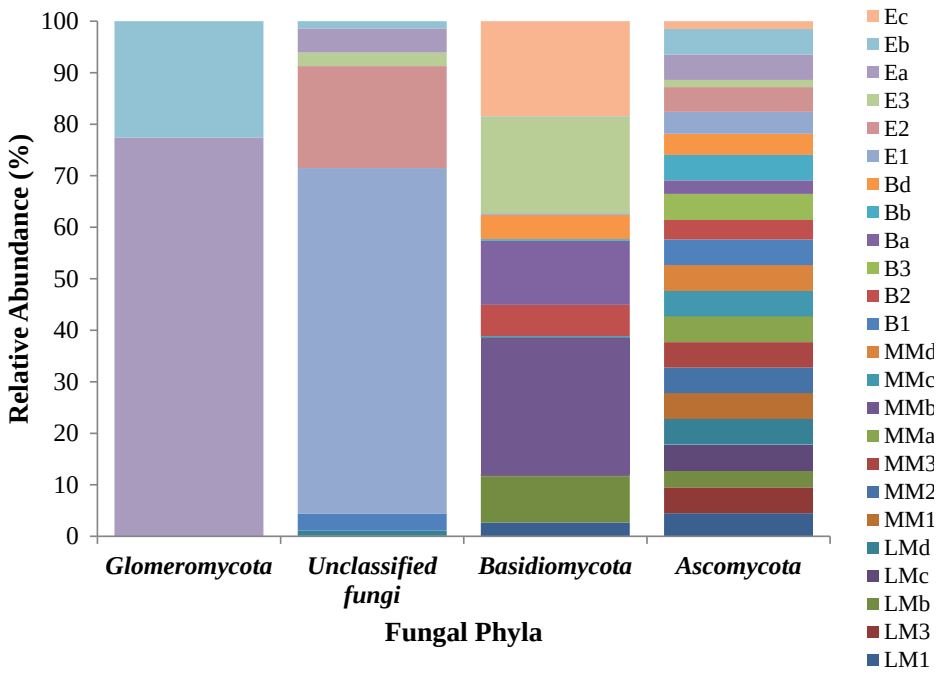

Figure 2. Taxonomic composition Analysis at phylum level.

they were present in 22 and 17 samples respectively, out of the 24 samples analyzed. The class *Dothideomycetes* was the most dominant in the water samples, representing 99.7% relative abundance in LMd at 81.9°C, 81.5% in MMd at 43.9°C and 37.3% in Bd at 84.6°C. In the microbial mats the class *Dothideomycetes* accounted for 54% in sample LM3 at 76.3°C, 98% in MM3 at 37.9°C, 89% in MM1 at 43.8°C, 98% in B3 at 54°C, 70% in B1 at

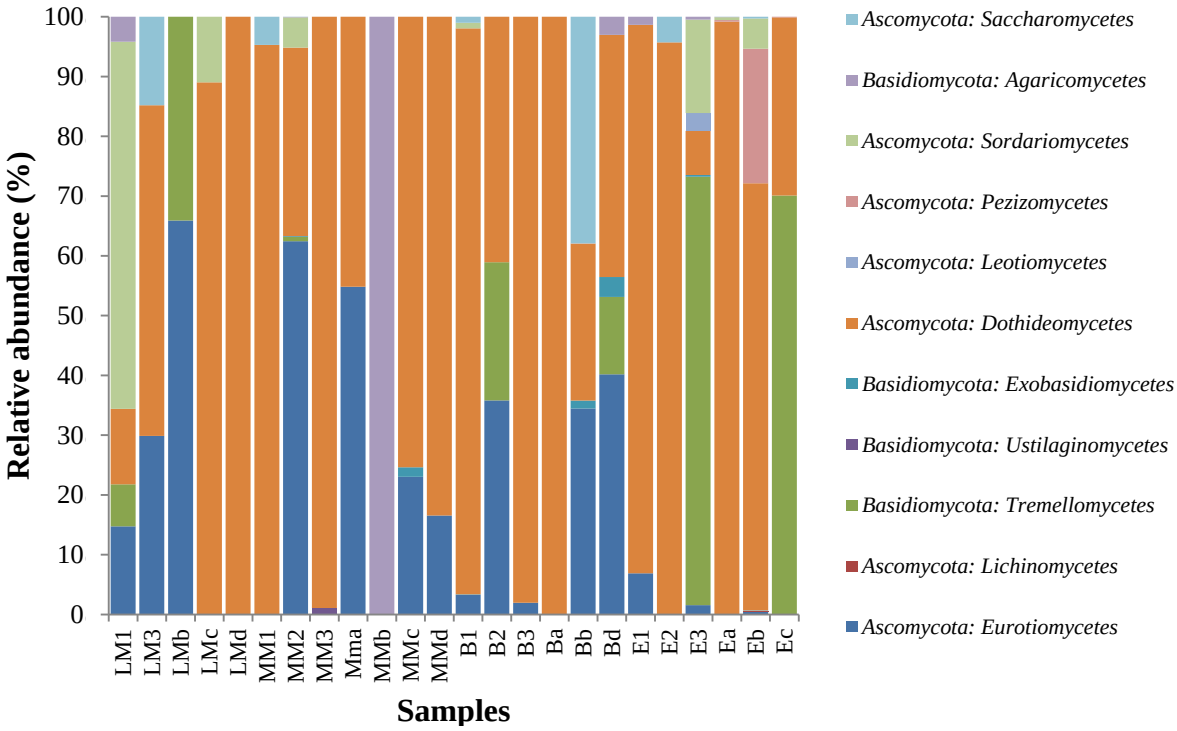

Figure 3. Taxonomic composition analysis at class level.

84.6°C, 91% in E2 at 44.7°C and 76% in sample E1 at 45°C. The percentages of the class *Dothideomycetes* in the wet sediments also varied. The highest value was recorded in sample Ea at 45°C with 85.6%. LMc recorded 69.9% at 76.3°C, Eb 60% at 44.7°C, 56% in sample MMc at 37.9°C and 52% in sample Ba at 84.6°C**.** The class *Eurotiomycetes* was also present in the water samples at 16% in MMd at 43.8°C, and 37% in Bd at 84.6°C (Figure 3).

Sequences from *Ascomycota* matched 16 known orders, with *Pleosporales* being the most diverse as it had six families affiliated to it. The most abundant fungal order was *Capnodiales* in little Magadi and lake Bogoria accounting for 99.8% in sample LMd at 81.9°C and 52.7% in sample Ba at 84.6°C respectively. The order *Dothideales* accounted for 95 % in sample MM1 at 43.9°C in lake Magadi and the order *Pleosporales* had a percentage of 91.6% in sample E2 at 44.7°C. Ascomycota phyla had twenty seven (27) families with *Davidiellaceae* being the most abundant and diverse family (99.8%) in sample LMd at 81.9°C, *Dothioraceae* (95%) in sample MM1 at 43.8°C, *Sporormiaceae* (91%) in sample E2 at 44.7°C, *Didymellaceae* (68 %) in sample MMd at 43.8°C, and *Onygenaceae* (55%) in sample MM2 at 41°C.

Out of the 62 genera detected in this study, the dominant genera were *Cladosporium* (99.79% in LMd, 52.79% in Ba), *Aureobasidium* (79.88% in MM1), *Aspergillus* (34.37% in Bb), *Penicillium* (65.90% in LMd), *Westerdykella* (91.62% in E2 and 79.40% in Ea), *Epicoccum* (42.76% in B1), *Debaryomyces* (12.67% in LM3), *Auxarthron (*16.18% in MMd) and among other varied percentages in many of the samples. Most genera were recorded from Lake Elmenteita in samples Ea at 45°C, Eb at 44.7°C and E3 at 33.8°C with varying percentages. The common fungal species were *Cladosporium* sp. (83.08% in LMd), *Cladosporium cladosporioides* (17.90% in Ba), *Pleosporales* sp. (86.07% in MM3), *Aureobasidium pullulans* (79.88% in MM1) and *Aspergillus oryzae* (35.02% in B2). *Cladosporium* sp. was present in 17 samples, *C. cladosporioides* in 14 samples, *Pleosporales* sp. in 13 samples A. *pullulans* in 13 samples and *Aspergillus oryzae* in 9 samples out of the 24 samples sequenced in this study.

Basidiomycota in hot springs of soda lakes

The distribution of *Basidiomycota phylum* was diverse within the samples, accounting for 100% in sample MMb at 41°C, 71% in sample E3 at 33.8°C and 70% in sample Ec at 33.8°C while the rest of the samples had relative abundances of below 50%. Sequences matching with *Basidiomycota* were affiliated to the following classes; *Agaricomycetes*, *Exobasidiomycetes*, *Tremellomycetes* and *Ustilaginomycetes* (Figure 3). *Tremellomycetes* was the most abundant and diverse, representing 70.6%

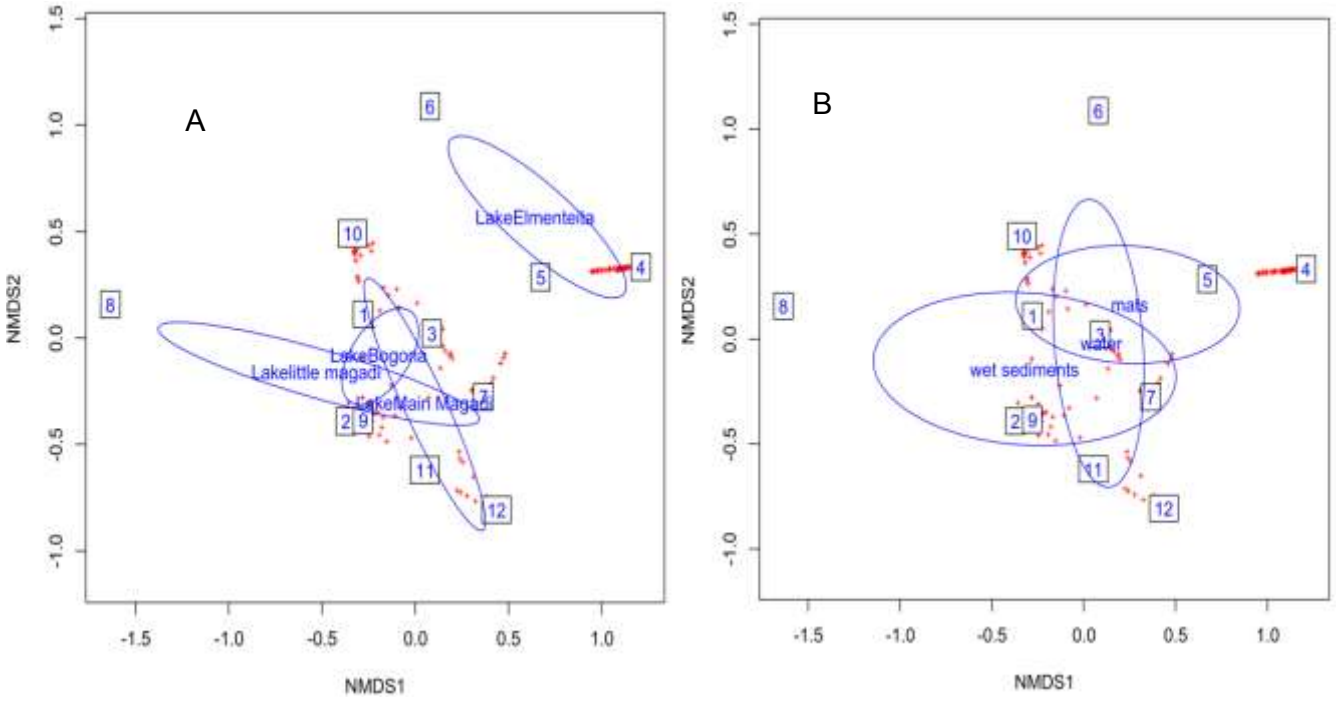

Figure 4. Non-metric dimensional scaling (NMDS) based on Bray Curtis dissimilarities between microbial compositions within various samples. A and B represents different lakes and sample types of each site respectively. References 1, 2 and 3; 4, 5 and 6; 7, 8 and 9; and 10, 11 and 12 represents mats, sediments and water samples from lake Bogoria, Elmenteita, Little Magadi and Magadi, respectively.

relative abundance in E3 at 33.8°C and 69.9% in sample Ec at the same temperature. *Agaricomycetes* recorded a relative abundance of 100% in sample MMb at 41°C. Sequences from *Basidiomycota* matched 9 known orders, with *Agaricales* being the most abundant recording 100% in sample MMb at 41°C. *Malasseziales* and *Tremellales* were the diverse orders as they were present in seven samples out of the possible 24 samples analyzed in this study. *Malasseziales* recorded 3.1% in sample Bd at 84.6°C while *Tremellales* had 70.5% in sample E3 and 70.05% in sample Ec both at 33.8°C.

At family level, OTUs were distributed in seven (7) fungal families with the most abundant (100%) belonging to *Lyophyllaceae* in sample MMb at 41°C, *Tremellaceae* (33%) in sample LMb at 67.9°C, *Lachnocladiaceae* (2.8%) in sample Bd at 84.6°C and *Malasseziaceae* (1.3%) in sample Bb at 77.7°C. Out of the 62 genera in this study, few were affiliated to *Basiodiomycota* phyla. This included *Termitomyces* (100%) in sample MMb at 41°C, *Rhodotorula* (47.2%) in sample Ba at 84.6°C, *Tremella* (32.95 %) in sample LMb at 67.9°C and *Malassezia* (1.3 %) in sample Bb at 77.7°C. Among the dominant species were; *Termitomyces* sp. (100 %) in sample MMb at 41°C, *Tremella aurantialba* (25%) and *Tremella encephala* (7.95%) both in sample LMb at 67.9°C, *Dioszegia hungarica* (1.7%) in sample MM2 at 41°C and *Malassezia globosa* (1.25%) in sample MMc at 37.9°C.

Glomeromycota and *unclassified fungi* in hot springs of soda Lakes

The phylum *Glomeromycota* was present only in samples Ea at 45°C and Eb at 44.7°C with 0.4 and 0.1% relative abundances respectively. The proportion of unclassified fungi was relatively small and was recorded in eight samples only with the highest relative abundance in sample E1 (13% at 45°C) and sample E2 (4% at 44.7°C) with the rest having relative abundances below 1% (Figure 2). Notably was the presence of order *Mortierellales* (0.07%) from the subkingdom *Incertae sedis* in sample Eb at 44.7°C.

Relationships between fungal communities and environmental variables

To test which environmental or geographical parameters correlated with community dissimilarity, a non-metric dimensional scaling (NMDS) plot was drawn for the sampling sites or lakes (Figure 4A) and sampling types (Figure 4B). These showed that the sample types had less influence on the fungal communities than temperature and/or the sampling sites, supporting environmental variation as the major determinant of fungal community structure. The Bray-Curtis clustering indicated a tendency of the communities to group by sample types

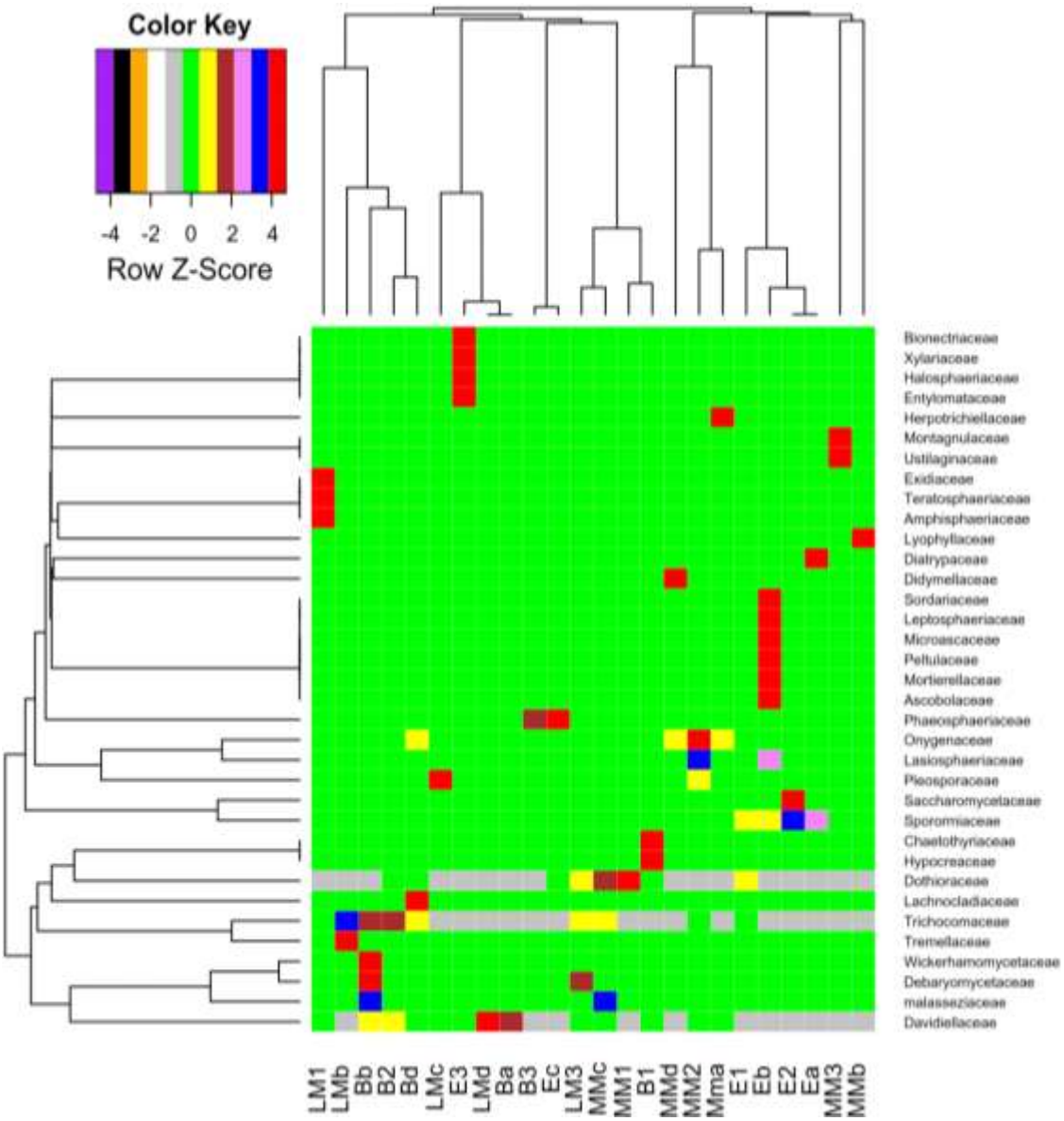

Figure 5. Hierarchical clustering of 18S rDNA samples collected from the four hot springs under investigation. Family level was chosen to be used in hierarchical clustering to assess the relationships between samples and taxa.

but differed in temperature and sampling sites. As the sampling sites were in different climatic regions, the changes in salinity, temperature and latitude appeared to have contributed to these differences.

Hierarchical clustering of various samples and fungal taxa at family level revealed the most dominant families to be *Entylomataceae*, *Halosphaeriaceae*, *Xylariaceae* and *Bionectriaceae* in microbial mats (Sample E3) at lake Elmenteita at 33.8°C and the families *Sordariaceae*, *Leptosphaeriaceae*, *Microascaceae*, *Peltulaceae*, *Mortierellaceae* and *Ascobalaceae* in wet sediments (Sample Eb) at the same lake at a temperature of 44.7°C. Lake Elmenteita was found to harbor the most dominant fungal taxa as compared to the other three. This could be attributed to the lower temperatures recorded during sampling that were favorable for the growth of fungi (Figure 5).

The phylogenetic diversity of fungi revealed in this study is relatively low compared to that of studies done on terrestrial or marine habitats like soils, plants and mangroves. Although a total of 2,179 OTUs were recorded in this study there was a distinct discrepancy in the number of OTUs per sampling site. Notably lake Elmenteita had the highest number of fungal OTUs at 1,196 followed distantly far by lake Magadi with 394 OTUs (Table 2).

DISCUSSION

There has been significant interest in finding life in extreme environments with high temperatures, salinity and pH like hot springs and soda lakes. To the best of our knowledge, this is the first report on the use of 454-pyrosequencing approach to investigate fungal diversity and community structure at different temperature gradients along the flow of hot springs of four Kenyan soda lakes. This ecological study aimed at examining fungal indicators of life in such extreme environments and being the first study to be done on hot springs of soda lakes, results here were compared to soils, hypersaline environments and sediments and not necessarily from soda lakes.

Sequences analyzed in this study revealed that the majority of recovered fungal sequences belonged to the Domain *Eukaryota* and comprised of the phyla *Ascomycota* (83.3%), *Basidiomycota* (15.8%), *Glomeromycota* (0.02%) and unclassified *fungi* (0.9%) which represented only a small proportion of the fungal communities. These results are in agreement with findings from Schadt et al. (2003) that found a large proportion of the members of the phylum *Ascomycota* in 125 cloned fungal sequences from Tundra soils. Similar studies on hypersaline environments done by Santini et al. (2015) on fungal communities found that 73% of the total OTUs were dominated by members of the phylum *Ascomycota* (52 to 100%) with minor contributions from the phylum *Basidiomycota*. Contrary to many reports on hypersaline environments, Singh et al. (2011) and Bass et al. (2007) found Basidiomycete yeasts to be the most dominant fungal forms in deep-sea environments. Generally members of the phylum *Ascomycota* occur naturally in all land ecosystems worldwide. Chytridiomycota, a phylum of fungi distinguished by having zoospores were evidently missing from the sequences obtained in this study. This observation is similar to the findings of a previous study on frequency and distribution of zoosporic fungi from moss covered and exposed forest soils that reported members of the phylum *Chytridiomycota* could also be found in freshwater or wet soils, with most species being infrequent and scarce to rare (Letcher and Powell, 2001; Letcher et al., 2004). Members of *Chytridiomycota* phylum are the simplest and most primitive Eumycota. Although many ecotypes are adapted to extreme environmental conditions, most members are ubiquitous in many ecosystems, especially in cool, moist soils and freshwater habitats that are rich in organic matter. This explains why the phylum *Chytridiomycota* could not be detected in saline environments with high temperatures and pH as these conditions proved harsh for their survival. However, a previous study that applied illumina DNA sequencing analysis of samples collected from the hot springs of Lake Magadi and Little Magadi showed members of Chytridiomycota to represent only a small proportion of the hot spring fungal communities (Kambura et al., 2016).

In this study, the most commonly identified classes within the phylum *Ascomycota* were *Sordariomycetes* and *Dothideomycetes*. The class *Sordariomycetes* had seven orders and nine families affiliated to it while the class *Dothideomycetes* had 3 orders and nine families. These data are consistent with a previous study on fungal communities in the deep-sea sediments of the Pacific Ocean (Xu and Luo, 2014) and that of diversity and distribution of fungal communities in marine sediments of Kongsfjorden, Svalbard, (Tao et al., 2015) that found the two classes *Sordariomycetes* and *Dothideomycetes* to be the most diverse and abundant. The classes *Sordariomycetes* and *Dothideomycetes* are so far the largest and most phylogenetically diverse classes within the phylum, *Ascomycota* (Kirk et al., 2008). The members are a heterogeneous group of fungi that subsist in majority of niches where fungi can be found.

The dominant genera were *Cladosporium, Aureobasidium, Aspergillus*, *Penicillium*, *Westerdykella, Epicoccum, Debaryomyces*, *Auxarthron* and *Malassezia*. The common fungal species were *Cladosporium* sp., *C. cladosporioides, Pleosporales* sp., *A. pullulans* and *A. oryzae*. Kambura et al. (2016) also found that *C. cladosporioides* species were unique to sediment samples that were collected at 83.6°C from the hot springs of Little Magadi. In the same study, Kambura et al. (2016) observed that sediment samples collected at 81°C had *Aspergillus* within the phylum *Ascomycota* as the most abundant species. This is also similar to previous studies in hypersaline waters of salterns that revealed different species of *Aspergillus, Penicillium* and diverse non-melanised yeasts (Gunde-Cimerman et al., 2005). Studies done by Razieh et al. (2015) indicated that most strains isolated from coastal waters of the southern Caspian Sea, belonged to the genus *Cladosporium*. Also, Damare et al. (2006) showed that the genera *Penicillium*, *Aspergillus* and *Cladosporium* were the most abundant in aquatic environments. Jaouani et al. (2014) isolated fungi belonging to the genera *Cladosporium, Alternaria, Aspergillus, Penicillium, Ulocladium, Engyodontium* and *Cladosporium cladosporioides* that were able to grow in media containing 10% of salt with an initial pH 10 from Sebkha El Melah, a Saharan Salt Flat in Southern Tunisia. A study done by Purnima et al. (2011) on the phylogenetic diversity of culturable fungi from the deep-sea sediments of the Central Indian Basin grouped the fungal microorganisms into seven (7) clusters belonging to *Aspergillus*, *Sagenomella* sp, *Exophiala* sp, *Capronia* sp, *Cladosporium*, *Acremonium* sp. and *Tritirachium* sp. Another study that used morphological and molecular techniques to identify a series of halotolerant fungi from hypersaline environments of solar salterns revealed 86 isolates of 26 species from salt ponds, which were identified as *C. cladosporioides*, nine *Aspergillus* sp, five *Penicillium* sp. and the black yeast *Hortaea werneckii* (Cantrell et al., 2006). In this study, most of the fungal

taxa such as *Aspergillus*, *Cladosporium* and *Penicillium* species are derived from terrestrial habitats like soils. This could be attributed to previous run off waters from adjacent areas that may have brought large numbers of terrestrial fungi in form of spores and fungal hyphae into the hot spring rivulets. Therefore, the fungi detected in this study may have originated from other environments and adapted to saline conditions, high temperatures and alkaline pH by developing effective strategies to tolerate stress in the hot springs. Due to this adaptability, the fungal groups could be candidates for exploitation in search for potential industrial products.

Conclusion

Study of fungi has been given little attention compared to other microorganisms like bacteria and archaea. Therefore, the findings in this study will be of high significance in the field of mycology. The results obtained using high-throughput analysis indicate that sediments, mats and water from the studied hot springs of soda lakes in Kenya are important niches that harbor unexpectedly high richness of fungal species, most of which possibly originated from terrestrial environments. However, the ecological roles of these fungi and their adaptive mechanism remain poorly investigated. It is also unclear if these fungi are actively growing in these environments or being dormant propagules (spores) that are washed into the sediments, microbial mats and water during the rainy seasons. Therefore, a combination of different technologies including traditional culture-based method, metagenomics, metatranscriptomics and meta-proteomics may help to answer these pending questions and may reveal the functions of the genes present in these extreme environments. The use of these technologies will have a huge impact on the functions of fungal communities in the ecosystem of Kenyan soda lakes and may also serve as a useful community model for further ecological and evolutionary study of fungi in these extreme environments.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ABBREVIATIONS

ITS, Internal transcribed spacer; **OTUs,** operational taxonomic units; **DNA,** deoxyribonucleic acid; **NMDS,** non-metric dimensional scaling**; QIIME,** Quantitative Insights into Microbial Ecology.

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