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Molecular diversity of plankton in a tropical crater lake switching from hyposaline to subsaline conditions: Lake Oloidien, Kenya

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Abstract Salinity in the climate sensitive tropical endorheic crater lake Oloidien (Great African Rift Valley, Kenya) decreased from hyposaline to subsaline conditions during the period 2010–2015. The change in salinity was accompanied by a pronounced change in planktonic life forms—from blooms of the cyanobacterium *Arthrospira* supporting tens of thousands of Lesser Flamingos to highly diverse communities of cyanobacteria and algae which do not sustain the consumer birds. Besides the well-known macro- and microscopic lake life, a hidden diversity of microorganisms was detected using molecular methods. SSU rRNA gene clone libraries and data from Ilumina Miseq sequencing of samples collected at the two contrasting stages revealed distinct and highly diverse microbial communities. Different bacterial clades dominated the two samples. In 2011, Firmicutes (class Bacilli) whose origin was the fecal waste of birds were the dominant group. However, the Cyanobacteria and Chloroflexi were the most prevalent in 2015. From the microbial eukaryote samples obtained in 2011, rotifers and ciliates that feed on *Arthrospira* and rich bacterial food dominated the plankton, while the cryptophytes were the most prevalent in 2015. On the two occasions, a mixture of organisms previously not known to occur in saline or in freshwater habitats was found.

Keywords Alveolata · Bacteria · Chlorophyta · Cyanobacteria · Fungi · Lesser Flamingo · Ilumina Miseq sequencing · SSU rRNA gene clone library · Rural water resources

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Introduction

Variation in salt concentration in aquatic environments has been shown to promote the diversity of microorganisms (Wilson et al., 2004; Buchheim et al., 2010). Climate responsive endorheic lakes of the tropics exhibit considerable variation in their hydroecology over long- or short-term scales—from phases of being flooded with freshwater to saline or even dried out (Verschuren, 2003; Schagerl & Renaut, 2016). Fluctuations in climate determine the balance of inflow and

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evaporation. In the case where evaporation exceeds inflow, minerals become more concentrated and salinity increases. On the converse, the dilution effect of increased freshwater inflows reduces the water salinity (Beadle, 1974; Schagerl & Renaut, 2016). The food web and biodiversity patterns of tropical endorheic lakes are usually a reflection of the salinity conditions (Wood & Talling, 1988; Kebede, 2002). Cases of drastic changes in food web structure and biodiversity were observed in soda lakes of East Africa (Krienitz & Kotut, 2010; Krienitz et al., 2016a). Under high salinity conditions, the community of primary producers is dominated by the cyanobacterium *Arthrospira fusiformis*(Voronikhin) Komárek & J.W.G. Lund, which supports spectacular populations of the flagship primary consumer, the Lesser Flamingo (Brown, 1959). However, the intervention of diverse factors can lead to the collapse of the mass development of *Arthrospira* (Melack, 1988; Peduzzi et al., 2014). One important factor is the drop in salinity to subsaline or fresh conditions. Consequently, based on food availability, conditions in the lakes change from flamingo to non-flamingo stages (Vareschi, 1978, 1982; Kaggwa et al., 2013; Krienitz et al., 2016a, b). We used the opportunity presented by this event to compare the molecular diversity of plankton in a soda lake in a stage when flamingos joined the habitat for feeding and in a stage when flamingos left the lake because of a deficit of edible microphytes Fig. 1. We analyzed the diversity of bacteria including cyanobacteria, algae, alveolates, and fungi in Lake Oloidien in these two contrasting ecological stages.

Lake Oloidien is one of the model water bodies selected for lake-based climate reconstruction in East Africa (Verschuren, 2003). It is a eutrophic crater lake in close vicinity to the freshwater lake Naivasha in the Central East African Rift Valley (Kalff & Watson, 1986; Ballot et al., 2009). Paläolimnological pigment analyses of the sediments of Lake Oloidien revealed shifts from freshwater to soda-lake conditions and vice versa as evidenced by considerable changes in phytoplankton communities. The different salinity and lake depth phases were characterized by cyanobacteria, chlorophytes, or diatoms communities (Verschuren et al., 1999). During the period between $\sim$1870 and 1991, the lake depth fluctuated between 4 and 19 m while conductivity varied between 250 and 14,000 µS cm^{-1} (Verschuren et al., 2000). Consequently, lakes Oloidien and Naivasha went through periods of being united to form one lake to periods when the two became separate water bodies. The recent developments date back to the 1980s when a decline in water level led to a separation of Lake Oloidien from Lake Naivasha (Verschuren et al., 1999). The basin of Oloidien is hydrologically closed. Water losses are entirely via evaporation while a rise in lake water is brought about by rainfall and subsurface inflow from Naivasha through a permeable sill (Verschuren et al., 1999). Electrical conductivity measurements made immediately after the separation of the two lakes in 1980 recorded a conductivity value of 660 µS cm^{-1} (Kalff & Watson, 1986). Between 2001 and 2005, conductivity ranged from 3,890 to 5,270 µS cm^{-1} (Ballot et al., 2009). During 2006–2013, the conductivity increased to 5,900–9,300 µS cm^{-1} (Krienitz et al., 2013a). Since April 2013, a steady rise in water levels in the two lakes was maintained until the connection between the two was re-established. The net inflow of freshwater into Lake Oloidien resulted in a decline in conductivity to 2,100 µS cm^{-1} and the introduction of fish into Oloidien.

Lake Oloidien is located very close to human settlements. The vibrant village built by Kongoni Estate on the dusty ashes of the volcanoes of the surrounding landscape hosts a population that largely relies on the raw water of the lake for diverse domestic chores. Hence, Oloidien is not a typical remote flamingo lake protected by the law, but rather a site where the unique and archaic spectacle of thousands of pink birds meets the hustle and bustle of civilization.

In the present paper, we analyze the molecular diversity of the lake in 2011 when Lesser Flamingos fed on *Arthrospira fusiformis*-dominated phytoplankton (Krienitz et al., 2013a), and in 2015 when the birds had disappeared due to the massive dilution of the lake water by runoff water and inflows from Lake Naivasha (Krienitz et al., 2016a). The impact of these changes on the ecosystem and especially the food web will be discussed.

Materials and methods

Sampling site

Lake Oloidien is an endorheic warm polymictic crater lake that experiences top to bottom mixing every night (Verschuren et al., 2000). The main characteristics are

Table 1 Characteristics of Lake Oloidien (2001–2015)

Geographic position	00°49′00″S, 36°15′50″E
Altitude a.s.l.	1,885–1,887 m
Size	400–550 ha
Depth	4–7 m
Volume	1.0–1.5 × 10^7 m^3
pH	9.3–10.4
Conductivity	2.000–10.4000 mS cm^{-1}
Alkalinity	34.5–149 meq l^{-1}
P (total phosphorus)	0.4–1.0 mg l^{-1}
N (total nitrogen)	0.9–6.3 mg l^{-1}

as provided in Table 1 (as described by Verschuren et al., 2000; Ballot et al., 2009; Krienitz et al., 2013a; and supplemented by our own measurements).

As Lake Oloidien is located within the vicinity of the Kongoni human settlement, the two stages of the lake ecosystem were characterized by distinct lake resource use by people and their livestock subsisting in the lake's catchment. In 2011 when the flamingo population was high, ecotourism activities took the centre stage with bird lovers frequenting the lake to admire the breath-taking ornithological spectacle and to go on sightseeing boat rides. In 2015, the low water salinity coupled with the introduction of fish from Lake Naivasha led to the invasion of the lake by fishermen. The Maasai herdsman, who are constantly on the look for livestock watering opportunities, became frequent visitors. The low water salinity was also suitable for domestic laundry and the shoreline was always dotted with women washing clothes. However, cloth washing and livestock watering prevailed at both stages of the lake even at a salinity level of about 4 ppt—perhaps because of a lack of an alternative source of water.

Sampling

Samples selected for the present study were collected on January 22, 2011 (sample designated as OL2011) and February 06, 2015 (designated as OL2015). Salinity, conductivity and pH were measured directly in the field using a WTW Multiline P4 m (Wissenschaftlich Technische Werkstätten Weilheim, Germany). Water samples for laboratory analyses were collected from a few centimeters below the water surface using an appropriate sample bottle. Samples for microscopy were fixed with formaldehyde to achieve a final concentration of 1%. For molecular analyses, 1,000 ml of the fresh sample was filtered in the field through membrane filters with a pore-size of 0.6 µm (Schleicher & Schuell GmbH, Dassel, Germany), air-dried and transported to the laboratory in an ice-box.

Microscopy

Phytoplankton numbers were counted in sedimentation chambers (Hydro-Bios Apparatebau GmbH, Kiel, Germany) under an inverted microscope Eclipse TS 100 (Nikon Corporation, Tokyo, Japan) as described by Utermöhl (1958). The phytoplankton biomass was calculated by geometric approximations using the computerized counting program OPTICOUNT (Opticount, 2008). The specific density of phytoplankton cells was assumed to be 1 g cm^{-3}. Morphological examination of the phytoplankton taxa in the sample was carried out using a Nikon Eclipse E 600 light microscope with differential interference contrast. Only cyanobacteria, algae, and a dense population of the rotifer *Brachionus* were studied under the microscope and compared with molecular findings, whereas the other groups were analyzed exclusively by Ilumina Miseq sequencing and clone library analyses.

PCR and Illumina MiSeq

The filters were mechanically sliced into small pieces and rinsed with cell lyses-buffer AP1 (Qiagen GmbH, Hilden, Germany). Cells were disrupted with the help of glass beads (Carl Roth GmbH, Karlsruhe, Germany; 0.7 mm) in the Tissuelyser II (Qiagen GmbH, Hilden, Germany). Genomic DNA was extracted using the DNeasy Plant Mini Kit (Qiagen GmbH, Hilden, Germany) following the instructions given by the manufacturer.

PCR of microbial eukaryotes

Polymerase chain reactions (PCR) of microbial eukaryotes were performed using primers with barcode flanking the hypervariable V4 region of the 18S rRNA gene: 3NDf (Cavalier-Smith et al., 2009) with the reverse primer V4_euk_R2 (Bråte et al., 2010). PCRs were conducted in 20-µl reactions with 0.2 µM each of the primers, ~10 ng of template DNA, 1×

PCR buffer, and 2.5 U of Pfu DNA Polymerase (Promega, USA). The amplification program consisted of an initial denaturation step at 95°C for 2 min, followed by 30 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s, and a final extension of 72°C for 5 min.

PCR of bacteria

The V3–V4 hypervariable regions of the bacterial 16S rRNA gene were amplified using PCR with the universal primers 338F (5′-barcode-ACTCCTACGGGAGGCAGCA-3′) (Muyzer et al., 1993) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) (Caporaso et al., 2011). PCRs were conducted in 20-μl reactions with 0.2 μM each of the primers, ~10 ng of template DNA, 1 × FastPfu buffer, and 2.5 U of Pfu DNA Polymerase (Promega, USA). The amplification program consisted of an initial denaturation step at 95°C for 3 min, followed by 27 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 45 s, and a final extension of 72°C for 10 min.

PCR of cyanobacteria

A nested PCR approach was used for the amplification of 16S rRNA gene fragments from the cyanobacterial genomic DNA present in the sample from the year 2015 (OL2015). This technique has greater specificity than regular PCR (Zwart et al., 2005). A low number of PCR cycles (20) were used to avoid biased amplification of the 16S rRNA gene fragment. For the first PCR, the primers CYA106F (Nübel et al., 1997; Li et al., 2001) and R4R (Li et al., 2001) were used to selectively amplify long fragments of cyanobacterial 16S rRNA genes from the samples as described previously (Ballot et al., 2004). For the second PCR, forward CYA359F and reverse primer CYA781R (Nübel et al., 1997) were used. The following PCR protocol was employed for amplification of the V3–V4 region of the 16S rRNA gene: an initial denaturation at 94°C for 3 min, followed by 19 cycles at 94°C for 1 min, 55°C for 45 s and 72°C for 1 min, followed by a final extension of 5 min at 72°C.

PCR products were pooled and purified using the DNA gel extraction kit (Axygen, Hangzhou, China). The DNA concentration of each PCR product was determined using a Quant-iT PicoGreen double-stranded DNA assay (Invitrogen, Germany) and quality control was carried out on a TBS-380 Mini-Fluorometer (Turner Biosystems, Sunnyvale, CA, USA). Finally, amplicons of all samples were pooled in equimolar concentrations.

SSU rRNA amplification and sequencing on the Illumina MiSeq 2000 were done by following the standard protocols of EMP (Earth Microbiome Project) (Caporaso et al., 2012). Raw sequence data are available from NCBI's Sequence Read Archive under study accession number SRP077628.

Diversity and community structure analyses

QIIME default parameters (Caporaso et al., 2010) were used for quality filtering (reads truncated at the first low-quality base and excluded if: (1) overlap ≤10 bp while the coupled reads were assembled into one single sequence, (2) less than 80% of read length was consecutive high quality base calls, (3) more than one error was present in the barcode, (4) the length was less than 50 bases, (5) the singletons were removed to avoid erroneous sequences). We picked operational taxonomic units (OTUs) using open reference UPARSE version 7.1 (http://drive5.com/uparse/) clustering against the August 18, 2013 release of the microbial amplicon reads database filtered at 97% identity. Reads that did not match any sequences in the reference database at ≥97% identity were clustered de novo. Poor-quality sequences and suspected chimeras were checked using BLAST with sequence segments separately, and then using the chimera check program UCHIME (http://drive5.com/usearch/manual/uchime_algo.html). In order to determine OTUs, the SILVA database (Release 123) which contained high-quality 18S rRNA genes and 16S rRNA genes (Quast et al., 2013), was chosen as a template. Sequences were clustered into OTUs defined by 97% similarity. Sequence reads with an average of 439 bps from bacteria and eukaryotic library were generated after trimming the primer sequences from the beginning and end of the raw data. We randomly picked 24,664 bacteria sequencing reads, and 24,412 eukaryotes sequencing reads, from each sample by using a pseudo-random generator for a secondary comparison of community composition and structure among samples. Finally, in order to check the overview of the cyanobacteria diversity from OL2015, we constructed a separate cyanobacteria library of OL2015 by using the specific primers; sequence reads with 403 bps were generated. A total of 32,740

Table 2 Comparison of the estimated operational taxonomic unit (OTU) richness and diversity indices of the 18S rRNA, 16S rRNA gene libraries (Bacteria and Cyanobacteria) for clustering at 97% (3%) identity, as obtained from Illumina Miseq sequencing analysis of Lake Oloidien (2011 & 2015)

Sample ID	Reads	0.97					
		OTU	Ace	Chao	Coverage	Shannon	Simpson
OL2011 (microbial eukaryotes)	24,412	65	65 (65, 65)	65 (65, 65)	1.000000	2.71 (2.69, 2.73)	0.1281 (0.1253, 0.1308)
OL2015 (microbial eukaryotes)	24,412	91	92 (91, 98)	92 (91, 102)	0.999877	2.21 (2.19, 2.23)	0.2307 (0.2262, 0.2352)
OL2011 (bacteria)	24,664	181	186 (183, 196)	186 (182, 202)	0.999554	2.76 (2.74, 2.79)	0.188 (0.1835, 0.1925)
OL2015 (bacteria)	24,664	179	183 (180, 192)	183 (180, 196)	0.999635	3.44 (3.42, 3.46)	0.0674 (0.066, 0.0687)
OL2015 (cyanobacteria)	32,740	30	30 (30, 30)	30 (30, 30)	1.000000	2.05 (2.04, 2.07)	0.1627 (0.161, 0.1644)

cyanobacteria sequencing reads were introduced into our analysis. Rarefaction analysis and Good's coverage for the five libraries were also determined. Comparison of the estimated OTU richness and diversity indices of the 16S rRNA and 18S rRNA gene libraries for clustering at 97% (3%) identity was shown in Table 2, as obtained from the Miseq sequencing analysis in Lake Oloidien.

To compare the microbial organisms diversity in the two samples, the Alpha-diversity such as Chao value, Shannon index and the Simpson index were analyzed by Mothur version v.1.30.1 (Schloss et al., 2011).

18S clone library protocol

The Miseq library of OL2011 was dominated by "big organisms" especially from metazoan sequences, which likely resulted in the full coverage by metazoans in the 18S rRNA gene clone library. However, OL2015 demonstrated a remarkable diversity of eukaryotic community; therefore, we sought to gain an insight into eukaryotic genotypes of this sample, and subsequently constructed a full length of 18S rRNA gene clone library. Eukaryotic SSU rRNA genes were amplified by PCR with eukaryote-specific primers EukA and EukB (Medlin et al., 1988). Amplified rRNA gene products from several individual PCRs were pooled. The clone library was constructed according to Luo et al. (2009, 2011). The polymerase chain reaction was performed with an initial denaturation for 10 min at 95°C, followed by 35 cycles of 30 s at 94°C, 30 s at 55°C, and 1 min 30 s at 72°C, followed by a final extension of 15 min at 72°C. 100 µL PCR products were cleaned using a QIAGEN purification kit, and then cloned with the Cloning kit (pGEM-T, Promega) following the manufacturer's directions. Libraries were screened for the whole 18S rRNA inserted by PCR with M13 primers.

Full-length sequencing was done by ABI 3730 Sequencer with four conserved primers: two internal to the PCR products (570 F: 5′-CCA GCA GCC GCG GTA ATT C-3′; 905 F: 5′-GTC AGA GGT GAA ATT CTT GG -3′) and two targeted to the plasmid (M13F and M13R).

Poor-quality sequences and suspected chimeras were checked using BLAST with sequence segments separately, and then using the Chimera Check Program at Ribosomal Data Project II (Cole et al., 2003). The sequences that passed Chimeric screening were phylogenetically grouped and aligned using Clustal X v.1.83; alignments were manually checked by using the 'multicolor sequence alignment editor' of Hepperle (2003). Some ambiguously aligned positions have been removed manually. The phylogenetic analysis was performed subsequently and neighbor joining (NEIGHBOR) with the Kimura two-parameter correction algorithm was calculated by Phylip 3.62 package. Support of branches of the tree was obtained by bootstrapping 1,000 data sets. The recent proposed revision of classification of eukaryotes was used in the designation

of lineages of the phylogenetic tree (Adl et al., 2005). The 18S rRNA clone library sequence accession numbers are for Alveolates KX465142–KX465210, and for other organisms KX465211–KX465237.

Results

Environmental data and microscopy

Field measurements of key physical and chemical properties of the lake water carried out on January 22, 2011 yielded a conductivity of 5,900 μS cm^{-1} which is equivalent to a salinity of 3.6 ppt (hyposaline), and a pH of 10.4. Along the lake shores, about 70,000 Lesser Flamingos were observed. A total phytoplankton biomass of 172 mg l^{-1} fresh-weight that was mostly due to a dense population of *Arthrospira fusiformis* was recorded. Only few coiled filaments of *Anabaenopsis elenkinii* V.V. Miller and small colonies of *Microcystis aeruginosa* (Kützing) Kützing were detected microscopically. Furthermore, numerous individuals of the zooplankton *Brachionus dimidiatus* Bryce which had ingested small fragments of *Arthrospira* were observed.

Repeat field measurements of the above physical and chemical properties on 06 February 2015 recorded a conductivity of 2,100 μS cm^{-1}, corresponding to a salinity of 1.0 ppt (subsaline), and a pH of 10.0. On this occasion, no flamingos were present at Oloidien. A total phytoplankton biomass of 49 mg l^{-1} fresh-weight that was dominated by coccoid cyanobacteria and green algae was recorded. The taxa present during the study period are as listed in Table 3.

Molecular diversity

Comparison of the estimated operational taxonomic unit (OTU) richness and diversity indices of the 18S rRNA, 16S rRNA gene libraries (including bacteria and cyanobacteria) for clustering at 97% identity, as obtained from Illumina Miseq sequencing analysis of Lake Oloidien (2011 and 2015) was as shown in Table 2. A total of 24,664 valid bacteria reads from each sample were involved; 181 OTUs of OL2011 and 179 OTUs of OL2015 were obtained from both samples. Similar bacteria richness, indicated as similar Chao value, was found in both samples. The significant cyanobacteria diversity was shown from the Bacteria library of OL2015. Based on an overview of the cyanobacteria library of OL2015, a total of 30 cyanobacteria OTUs were traced. A total of 24,412 valid eukaryotic reads from each sample were involved, 65 OTUs of OL2011 and 91 OTUs of OL2015 were obtained from both Lake Oloidien samples through MiSeq sequencing analysis (Table 2). Richer eukaryotic diversity of OL2011 characterized by a higher Shannon index and lower Simpson index than those of OL2015 was found (Table 2).

Bacteria

Altogether, 13 different phyla of bacteria were found in the two samples from Lake Oloidien (Fig. 2a). Nine of them (Firmicutes, Cyanobacteria, Proteobacteria, Bacteroidetes, Chloroflexi, Actinobacteria, Acidobacteria, Verrucomicrobia, and Deinococcus–Thermus) were observed in both libraries, which comprised more than 90% of the total reads in every library (Fig. 2a). A total of 46 shared OTUs were obtained from both Lake Oloidien samples (Fig. 2b).

Although the taxonomy data obtained covered a broad spectrum of known bacterial phyla, the relative abundances of dominant bacterial phyla from both samples differed from each other significantly. Bacterial phyla obtained from OL2011 belonged to Firmicutes (72.49%), Proteobacteria (10.87%), Bacteroidetes (8.02%), Cyanobacteria (6.71%), Actinobacteria (0.93%), Fusobacteria (0.35%), Verrucomicrobia (0.13%), Chloroflexi (0.11%), Deinococcus–Thermus (0.07%), and Acidobacteria (0.03%). Firmicutes, which was the most abundant group (Fig. 2a), comprised mostly of the class Bacilli (70.84%) and Clostridia (1.65%). As shown in Table 4, Bacillales consisted mainly of unclassified (40.44%), *Bacillus* (10.8%) and *Oceanobacillus* (6.73%). The second most abundant phylum Proteobacteria was dominated by members of Gammaproteobacteria that contributed 6.28% of the total reads. The subdivision of Betaproteobacteria and Alphaproteobacteria contributed 3.62 and 0.78% of the total reads, respectively. The Deltaproteobacteria only contributed a very small fraction (0.04%) of the total reads. Bacteroidetes (8.02%) and Cyanobacteria (6.71%) were the third and fourth most abundant phyla, respectively, from OL2011.

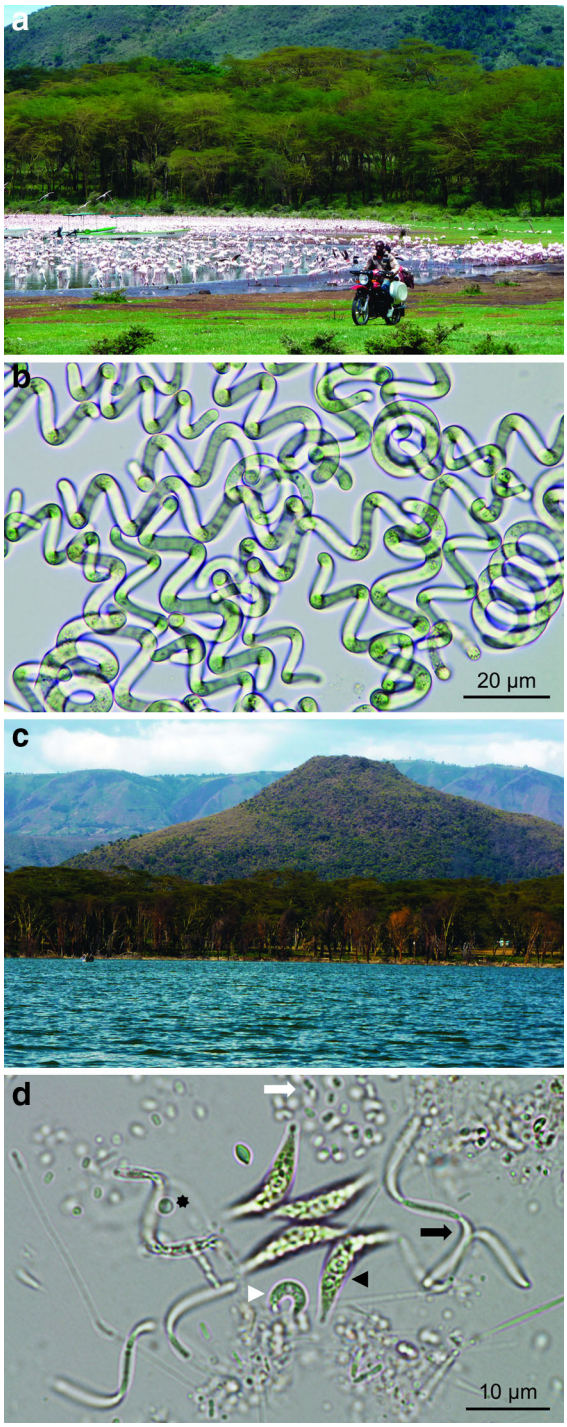
The bacterial groups from OL2015 comprised Cyanobacteria (61.05%), Chloroflexi (10.63%),

Table 3 Dominating and abundant taxa of phytoplankton in Lake Oloidien at the two sampling dates, 2011 (marked with asterisk) and 2015

Cyanobacteria
**Anabaenopsis elenkinii*
**Arthrospira fusiformis* (Voronichin) Komárek et Lund
Aphanocapsa delicatissima West et West
Aphanocapsa elachista West et West
Aphanothece smithii Komárková-Legnerova et Cronberg
Cyanodictyon imperfectum Cronberg et Weibull
Lemmermanniella sp.
Merismopedia punctata Meyen
Merismopedia tenuissima Lemmermann
**Microcystis aeruginosa* (Kützing) Kützing
Microcystis novacekii (Komárek) Compère
Raphidiopsis curvata Fritsch & Rich
Romeria gracilis (Koczwara) Koczwara ex Geitler
Spirulina subsalsa Oerstedt ex Gomont
Synechococcus sp.
Synechocystis sp.
Bacillariophycea
Anomoeoneis sphaerophora Pfitzer
Chlorophyta
Acutodesmus obliquus (Turpin) Hegewald et Hanagata
Botryococcus terribilis Komárek et Marvan
Chlamydomonas div. sp.
Chlorella div. sp.
Crucigeniella apiculata (Lemmermann) Komárek
Desmodesmus div. sp.
Dictyosphaerium ehrenbergianum Nägeli
Heynigia dictyosphaerioides C. Bock et al.
Hindakia fallax (Komárek) C. Bock et al.
Koliella sp.
Mychonastes homosphaera (Skuja) Kalina & Punčochářová
Oocystis sp.
Pectinodesmus pectinatus (Meyen) E. Hegewald et al.
Quadricoccus sp.
Raphidocelis subcapitata (Korshikov) Nygaard et al.
Indetermined phytoflagellates (Cryptophyta, Dinophyta, Chrysophyta)

Actinobacteria (6.72%), Proteobacteria (6.44%), Bacteroidetes (5.02%), Tenericutes (2.92%), Firmicutes (1.07%), Acidobacteria (1.06%), Verrucomicrobia (0.73%), Gemmatimonadetes (0.42%), and Deinococcus–Thermus (0.31%) phyla. Cyanobacteria were found to be the most abundant phylum. Chloroflexi, which was the second most abundant phylum, was mainly represented by Anaerolineales (7.84%), Caldilineales (1.03%), and unclassified Chloroflexi (Table 5). Members of Betaproteobacteria dominated the Proteobacteria phylum contributing 2.23% of the total reads. The subdivision of Gammaproteobacteria and Alphaproteobacteria accounted for 1.61 and 1.42% of the total reads, respectively.

Cyanobacteria (6.71% of all bacteria reads), which was the third most abundant group in library of OL2011 (Fig. 2a) comprised *Arthrospira*, which was the major taxa (4.55% of bacteria reads), *Anabaenopsis* (1.95% of bacteria reads), and a few reads related to *Microcystis* (<0.01% of bacteria reads) (Fig. 2e;



◀**Fig. 1** Lake Oloidien scenery and phytoplankton. **a** In 2011, dense flocks of Lesser Flamingo fringed the lake shore. A motorbike driver brings a washerwoman to her job site. **b** In 2011, the phytoplankton was dominated by a small-celled ecotype of the cyanobacterium *Arthrospira fusiformis*. **c** In 2015, the water level increased considerably, and no flamingos were at the lake. **d** In 2015, the phytoplankton was dominated by different cyanobacteria and green algae: *Aphanothece* (white arrow), *Raphidiopsis* (*black arrow*), *Raphidocelis* (*white arrowhead*), *Pectinodesmus* (*black arrowhead*), *Mychonastes* (*black star*)

Table 4). In contrast, Cyanobacteria (61.1% of all bacteria reads) were the most abundant taxon from the bacteria library of OL2015 (Fig. 2a), while 16 related OTUs were traced. On an account of the high diversity of cyanobacteria taxa of the sample OL2015, we constructed a cyanobacteria library with specific cyanobacteria primers (Fig. 2f). A total 30 OTUs where traced with 1.0 full coverage (Table 5). Among the Cyanobacteria, all the sequence reads were divided into the following 9 genera; *Synechococcus* (42.8%), *Spirulina* (27.8%), *Romeria* (12.4%), *Cyanobacterium* (11.8%), *Microcystis* (2.1%), *Anabaenopsis* (1.4%), and a few reads closely related to *Merismopedia*, *Geminocystis, Synechocystis*. Similarly, the top OTUs within the bacteria library also showed the significant predominance of *Synechococcus*, *Spirulina*, *Romeria* within Cyanobacteria (Table 5).

Microbial eukaryotes

The taxonomy data obtained covered a broad spectrum of known microbial eukaryote phyla (Fig. 2c). The microbial eukaryotes community from both samples differed significantly from each other. A total of 18 shared OTUs were obtained from both samples (Fig. 2d). Eight phyla of the microbial eukaryote communities were determined from OL2011, and 11 phyla from OL2015. The dominant phyla of OL2011 belonged to Metazoan (56.32%), Alveolata (16.93%), Fungi (8.99%), Stramenopile (8.58%), Cryptophyta (5.79%), Chlorophyta (2.89%), unclassified eukaryotes (0.4%), and Amoebozoa (0.1%). A total of 65 OTUs were detected in this sample with the large organisms (Metazoan) such as *Brachionus*, Arthropoda etc., being mostly traced. Alveolata consisted mainly of Ciliophora (16.93%). Ciliophora-related sequences fell into *Linostomella* (7.38%), *Vorticella* (4.11%), unclassified Conthreep (1.5%), *Didinium* (1.16%), *Ichthyophthirius* (1.15%), *Stokesia* (1.11%), and a few reads related to *Halteria* etc. Fungi were mainly represented by Ascomycota (8.08%), while still a few sequences reads

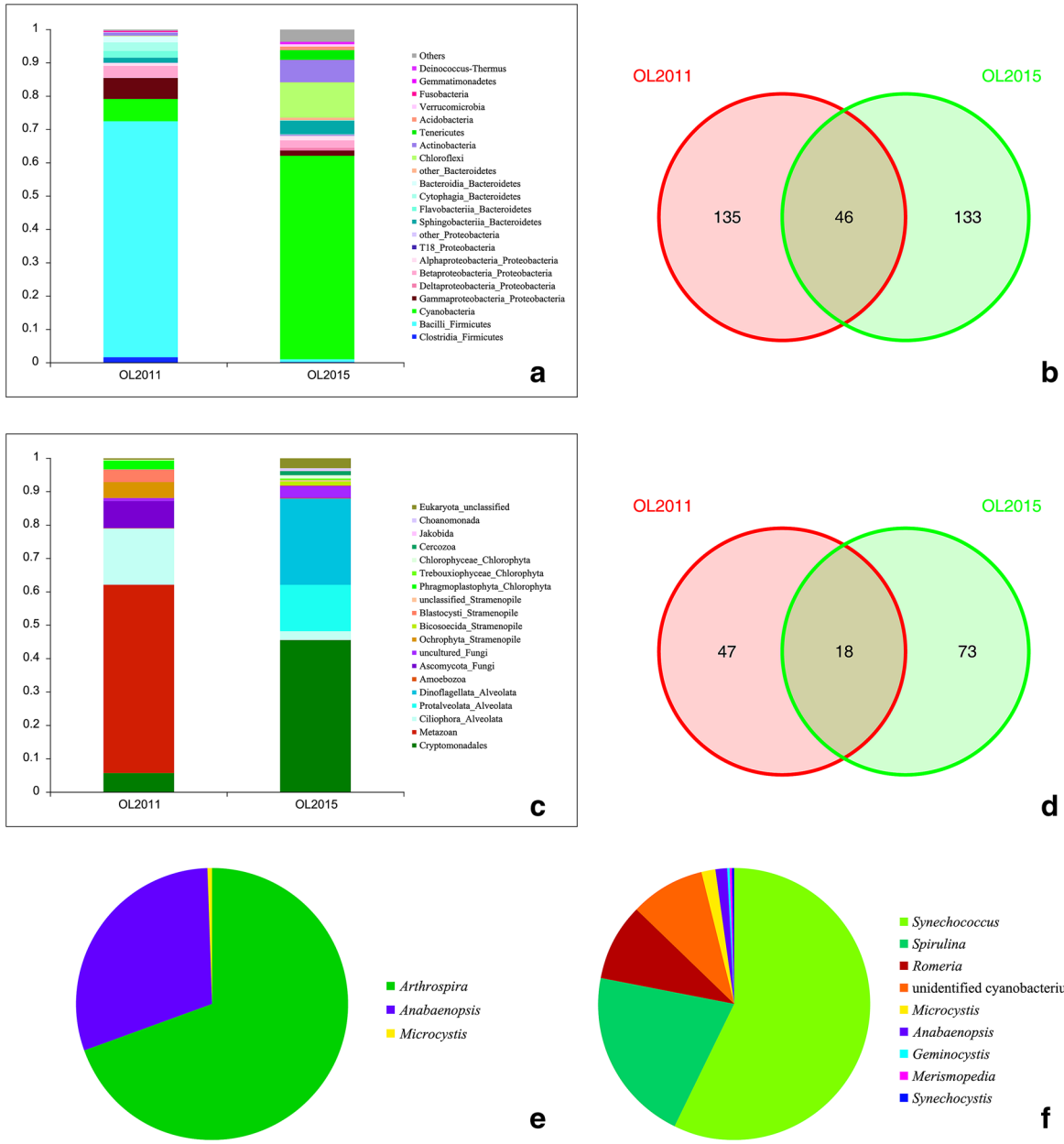


Fig. 2 Bacterial and microbial eukaryote composition and relative read abundance of the different planktonic communities in Lake Oloidien at two sampling dates. Sequences that could not be classified into any known group were assigned as unclassified. **a** Stacked column graph representing the relative abundance of the dominant high-level taxonomic phyla of bacteria. **b** Venn diagram showing the unique and shared operational taxonomic units (OTUs) (0.03 distance level) in the different Bacteria libraries in 2011 and 2015. **c** Stacked column graph representing the relative abundance of the dominant high-level taxonomic phyla of microbial eukaryotes. **d** Venn diagram showing the unique and shared OTUs in the different microbial eukaryotic libraries in 2011 and 2015. **e**, **f** Relative abundance of different genera of Cyanobacteria in 2011 (**e**) and 2015 (**f**)

were closely related to Basidiomycota, Cryptomycota, Glomeromycota, and uncultured fungi. Stramenopiles consisted of *Blastocystis* (3.76%), *Anomoeoneis* within Bacillariophyta (2.97%), *Poterioochromonas* within Chromulinales (1.34%), and an unclassified Ochrophyte. In Chlorophyta, except Embryophyta, few reads

Table 4 Average relative abundance of major groups of plankton OTUs in Lake Oloidien on January 22, 2011 **(OL2011)**, Kenya, based on Illumina Miseq Sequencing

Parent taxa	Closest match taxon (acc. no. NCBI)	Relative abundance (%) (represent. OTU)[a]	Similarity identity (%)	Origin	References
Bacteria					
Cyanobacteria					
Oscillatoriales	*Arthrospira platensis* IPPAS B-256 (**KX262886**)	4.55 (OTU164)	99	Lake Bodon, Chad	Unpublished
Nostocale	*Anabaenopsis elenkinii* (**KM020015**)	1.95 (OTU100)	99	SAG 252.80	Unpublished
Chroococcales	*Microcystis* sp. KLL-C019 **(KP726247)**	<0.01 (OTU196)	99	Lake Kinneret	Kaplan-Levy et al. (2016)
Other bacteria					
Bacillales	Uncultured bacterium clone TE1a05f12_13526 (**JQ368784**)	40.44 (OTU129)	99	FACE soil sample	Dunbar et al. (2012)
Bacillales	*Bacillus megaterium* (**KX129778**)	10.8 (OTU78)	99	Strain APFSG3isox	Unpublished
Bacillales	*Oceanobacillus* sp. 812B5_12ACASO (**KU644449**)	6.73 (OTU86)	100	Output sample of biogas plant BGA 005, Germany	Unpublished
Lactobacillales	*Lactococcus piscium* (**KT767809**)	5.22 (OTU133)	99	Milk	Unpublished
Lactobacillales	*Enterococcus hirae* (**KU353616**)	3.39 (OTU306)	99	Strain 080204	Unpublished
Pseudomonadales	*Pseudomonas deceptionensis* (**KR338996**)	1.94 (OTU108)	99	Strain DC5	Unpublished
Enterobacteriales	*Escherichia fergusonii* (**KX062000**)	1.71 (OTU269)	99	Strain CF5	Unpublished
Cytophagales	Uncultured bacterium clone MD14a11_12263 (**JQ373210**)	1.64 (OTU145)	99	FACE soil sample	Dunbar et al. (2012)
Microbial eukaryotes					
Cryptophyta					
Cryptophyta	*Guillardia theta* (**X57162**)	5.69 (OTU76)	99	–	Douglas et al. (1991)
Alveolata					
Ciliophora	*Linostomella* sp. cPFEU1 (**LN870136**)	7.38 (OTU133)	100	Tuscany, Italy	Rossi et al. (2016)

Table 4 continued

Parent taxa	Closest match taxon (acc. no. NCBI)	Relative abundance (%) (represent. OTU)[a]	Similarity identity (%)	Origin	References
Ciliophora	*Vorticella microstoma* **(JN120206)**	4.11 (OTU67)	98	Isolate 42Vmicrostoma52609wh	Sun et al. (2012)
Ciliophora	Uncultured ciliate clone PS05-108 **(DQ115965)**	1.5 (OTU97)	98	Polycyclic aromatic hydrocarbon-polluted soil	Unpublished
Ciliophora	Uncultured eukaryote clone 282A01 **(KJ925506)**	1.16 (OTU112)	100	Columbia River coastal margin	Kahn et al. (2014)
Ciliophora	*Ichthyophthirius multifiliis* **(KJ690572)**	1.15 (OTU124)	97	Isolate Ark9/1-1688	MacColl et al. (2015)
Ciliophora	Uncultured ciliate clone NPS05-2	1.11 (OTU34)	96	Polycyclic aromatic hydrocarbon-polluted soil	Unpublished
Stramenopiles					
Stramenopiles	*Blastocystis* sp. **(KT819588)**	3.76 (OTU83)	85	Thailand	Unpublished
Bacillariophyta	*Anomoeoneis sphaerophora* **(KJ011612)**	2.97 (OTU28)	96	Iowa, USA	Nakov et al. (2014)
Synurophyceae	*Poterioochromonas malhamensis* **(FN662745)**	1.34 (OTU121)	99	Strain DS	Jost et al. (2010)
Cercozoa					
Cercomonadida	*Paracercomonas* sp. Panama83 **(FJ790737)**	7.38 (OTU33)	99	–	Bass et al. (2009)
Fungi					
Dikarya	*Aspergillus penicillioides* **(DQ985960)**	4.3 (OTU77)	99	Strain 979	Vilela et al. (2007)
Dikarya	Uncultured *Cladosporium* clone ADVF10 **(KP968529)**	0.97 (OUT79)	99	Clinical eye swab	Unpublished
Dikarya	*Pseudocamarosporium* sp. CP-2016 **(KU754542)**	0.88 (OTU99)	99	Host: Pinus nigra	Unpublished

Total reads of bacteria = 24,664, total reads of microbial eukaryotes = 24,412

[a] The relative abundance of each representative OTU was determined from Bacteria and Eukaryotes, respectively

Table 5 Average relative abundance of major groups of plankton OTUs in Lake Oloidien on February 06, 2015 (**OL2015**), Kenya, based on Illumina Miseq sequencing

Parent taxa	Closest match taxon (acc. no. NCBI)	Relative abundance (%) (represent. OTU)[a]	Similarity identity (%)	Origin	References
Bacteria					
Cyanobacteria					
Chroococcales	Uncultured *Synechococcus* clone SP-B1-29 (**JF428826**)	15.71 (OTU68)	99	Shrimp pond sediment, India	Unpublished
Spirulinales	*Spirulina laxissima* SAG 256.80 (**KM019976**)	11.69 (OTU126)	99	Lake Nakuru, Kenya	Unpublished
Chroococcales	*Synechococcus* sp. CENA180 (**KC695872**)	8.94 (OTU267)	98	Cardoso Island, Brazil	Silva et al. (2014)
Chroococcales	*Synechococcus* sp. BE0807I (**FJ763789**)	7.64 (OTU 90)	99	Eutrophic freshwater, Great Mazurian, Poland	Jasser et al. (2010)
Oscillatoriales	*Romeria* sp. KLL-H-201 (**JQ819251**)	5.24 (OTU73)	98	Hula Nature Reserve, Israel	Unpublished
Cyanobacteria	Uncultured cyanobacterium clone FALLScyano02E02 (**DQ398202**)	4.68 (OTU284)	100	Falls Lake, North Carolina	Unpublished
Other bacteria					
Anaerolineales	Uncultured Chloroflexi bacterium (**GQ484146**)	7.84 (OTU24)	92	Intertidal thrombolites	Myshrall et al. (2010)
Caldilineales	Uncultured Chloroflexi bacterium clone MLS30 **JX240801**	1.03 (OTU89)	94	Coastal soil, India	Keshri et al. (2015)
Rhodocyclales	*Azoarcus* sp. enrichment culture clone N2 (**KP076660**)	1.08 (OTU25)	98	Large shallow freshwater lake, China	Ye et al. (2016)
Sphingobacteriales	Uncultured bacterium clone chfb1-63 (**HM050516**)	1.06 (OUT 310)	99	Lake Chaohu bacterioplankton sample	Unpublished
Acholeplasmatales	Uncultured *Acholeplasma* sp. FR838990	0.99 (OTU262)	95	Coal mines	Beckmann et al. (2011)
Microbial eukaryotes					
Cryptophyta					
Cryptophyta	*Guillardia theta* (**X57162**)	43.17 (OTU76)	99	–	Douglas et al. (1991)
Cryptophyta	*Hanusia phi* (**U53125**)	2.19 (OTU125)	90	CCMP 325	Unpublished
Chlorophyta					
Chlorophyta	*Desmodesmus denticulatus* (**KP726266**)	0.66 (OTU36)	99	Strain KLL-G003	Kaplan-Levyet al. (2016)
Chlorophyta	*Chlorella sorokiniana* (**KP771817**)	0.47 (OTU101)	100	Fresh water, China	Unpublished
Alveolata					
Dinophyta	Uncultured dinoflagellate clone LP80ME80 (**FJ903098**)	16.03 (OTU108)	87	Wall biomat 80 m deep in cenote La Pilita, Mexico	Unpublished
Dinophyta	*Pentapharsodinium dalei* (**JX262492**)	0.81 (OTU29)	93	Strain SCCAP K-1100	Orr et al. (2012)

Table 5 continued

Parent taxa	Closest match taxon (acc. no. NCBI)	Relative abundance (%) (represent. OTU)[a]	Similarity identity (%)	Origin	References
Dinophyta	*Esoptrodinium* sp. RP (**JQ439944**)	8.66 (OTU22)	97	USA	Fawcett & Parrow (2012)
Ciliophora	*Peritrichia* sp. aOmb2 **(LN869949)**	1.07 (OTU119)	100	Ombrone Stream, Italy	Rossi et al. (2016)
Ciliophora	*Vorticella microstoma* **(JN120206)**	0.87 (OTU67)	99	Isolate 42Vmicrostoma52609wh	Sun et al. (2012)
Protalveolata	Uncultured eukaryote clone KRL01E37 (**JN090897**)	7.48 (OTU12)	97	Lake Karla, Greece	Oikonomou et al. (2012)
Protalveolata	Uncultured eukaryote clone KRL01E25 (**JN090885**)	6.42 (OTU114)	99	Lake Karla, Greece	Oikonomou et al. (2012)
Incertae_Sedis	Uncultured eukaryote clone KRL03E06 (**KC315807**)	0.42 (OTU113)	96	Lake Karla, Greece	Nikouli et al. (2013)
Fungi					
Fungi	Uncultured fungus clone B26 (**JN054664**)	1.07 (OTU38)	83	Activated sludge from municipal wastewater treatment plant, Australia	Unpublished
Fungi	Uncultured *Paramicrosporidium* clone 415 **(KR131433)**	0.67 (OTU25)	83	Antarctic snow	Unpublished
Fungi	*Pseudomerulius curtisii* REH8912 (**GU187641**)	0.46 (OTU117)	80	Araucaria, Pinus, Rain Forest	Binder et al. (2010)
Fungi	Uncultured fungus clone B26 (**JN054664**)	0.45 (OTU73)	85	Australia	Unpublished
Fungi	Uncultured fungus clone B26 (**JN054664**)	0.42 (OTU115)	85	Australia	Unpublished
Cercozoan					
Cercozoa	*Paracercomonas* sp. Panama107 **(FJ790735)**	1.07 (OTU87)	98	Strain Panama107	Bass et al. (2009)
Stramenopiles					
Bicosoecida	Uncultured bicosoecid clone CH1_2B_3 **(AY821965)**	0.9 (OTU129)	94	fresh water filtrate (0.22–5 µm) Chevreuse, France	Slapeta et al. (2005)
Choanoflagellida					
Choanoflagellate	Uncultured choanoflagellate clone CV1_B2_17 **(AY821949)**	0.59 (OTU39)	93	Fresh water clay-sand sediment, France	Slapeta et al. (2005)

Total reads of bacteria = 24,664, total reads of microbial eukaryotes = 24,412

[a] The relative abundance of each representative OTU was determined from Cyanobacteria and Eukaryotes, respectively

were related to Chlorophyceae (0.2%). Cryptophyta was mainly represented by *Guillardia* (5.69%), and a few reads close to *Hanusia*.

The dominant phyla of OL2015 belonged to Cryptophyta (45.57%), Alveolata (42.36%), Fungi (3.64%), unclassified eukaryotes (2.92%), Stramenopile (1.74%), Chlorophyta (1.45%), Cercozoa (1.25%), Choanoflagellida (0.62%), Jakobida (0.27%), Amoebozoa (0.18%), and Metazoan (0.02%). 91 OTUs were traced in OL2015. The average relative abundance of major groups of plankton OTUs in Lake Oloidien is as shown Table 5. The most abundant group in this sample, the Cryptophyta were mainly represented by *Guillardia* (43.17%), *Hanusia* (2.19%) and unclassified cryptophytes. Alveolates, which consisted mainly of Protalveolata, Dinoflagellates, and Ciliophora, were the second most abundant group. Protalveolata comprised two groups: Perkinsidae (7.48%) and Colpodellida (6.42%). Dinoflagellate-related sequences consisted of uncultured dinoflagellates (16.03%), *Esoptrodinium* (8.66%), *Pentapharsodinium* (0.81%), *Peridinium* and unclassified Dinophyta. Within the Ciliophora, *Peritrichia* (1.07%), *Vorticella* (0.87%), Haptori (0.22%), and a few reads related to *Cryptocaryon*, *Cyrtolophosis*, *Halteria*, and *Cyclidium* were traced. Fungi's presence was dominated by unclassified (3.07%), and a few reads related to Chytridiomycota and Ascomycota. Among the members of Stramenopiles, the most common reads detected belonged to the Bicosoecida (0.9%) and a few reads related to *Ochromonas*, *Paraphysomonas*, Eustigmatales, and Chromulinales. Chlorophyta consisted mainly of organisms belonging to Chlorophyceae (*Desmodesmus*, *Mychonastes*, and *Chlamydomonas*) and Trebouxiophyceae (*Chlorella*). Cercozoa comprised mostly of *Paracercomonas* (1.07%), and a few unclassified reads. Choanoflagellida was only represented by uncultured choanoflagellates (0.59%). Jakobida and Amoebozoa were mainly represented by the genera *Jakoba* (0.27%) and Leptomyxida (0.18%), respectively.

Microbial eukaryotes of OL2015 discovered by 18S clone library

As the Miseq library of OL2011 was dominated by Metazoan sequences and OL2015 demonstrated a remarkable diversity of eukaryotic community, we sought to gain an insight into eukaryotic genotypes of sample OL2015, and subsequently constructed an 18S rRNA gene clone library. In total, 96 clones of the SSU rRNA genes comprising 23 of phylotypes were completely sequenced (Figs. 3, 4). The chimera detection software did not uncover any putative chimeras. The sample OL2015 revealed a rich diversity of seven phylogenetic groups: Alveolata (Fig. 3), Fungi, Cryptophyta, Stramenopiles, Chlorophyta, Cercozoa, and Choanoflagellida (Fig. 4). The Alveolata, which contributed 69.8% of all clones, was found to be the most diverse group. Among members of Alveolata, 61% of related clones belonged to Ciliophora; the Dinophyta clade (34.3% of Alveolata-related clones) was another major group, except that three clones were closest to *Colpodella* (Colpodellidae). The clones with ≥97% similarity to ciliophora clades such as *Opisthonecta*, *Tokophrya*, *Vorticella*, and *Cyclidium* were traced, and others clustered into uncultured Ciliophora clones. Three clones had 97% similarity to *Esoptrodinium* (Dinophyceae), while twenty clones clustered into unknown dinophyte clones.

Chlorophyta, took up 21.7% of all clones and formed five different sub-clades (Fig. 3b). Our analysis revealed the presence of genotypes of coccoid chlorophytes (99% similarity) such as *Acutodesmus*, *Chlorella*, *Mychonastes*, *Chlamydomonas*, and *Scenedesmus*, which were also recorded by microscopic observations. Nine clones were closely related to *Acutodesmus obliquus* (Turpin) Hegewald & Hanagata (strain SAG 276-3a) with 99% similarity, dominated in this library sample. Five fungi-related clones were traced. Stramenopile, which was the fourth major group, was composed of *Pseudocharaciopsis minuta* (A. Braun) Hibberd (97% similarity), uncultured stramenopiles clones related to those from Lake Nakuru, and uncultured Bicosoecida clones. Three clones with a 99% similarity to *Guillardia theta* D.R.A. Hill & R. Wetherbee and NKS170 from Lake Nakuru, clustered into Cryptophyta. One clone had a 96% similarity to *Paracercomonas saepenatans* Vickerman within Cercozoa. One clone with 92% similarity to *Eimeriidae* clone was grouped into Choanoflagellidae.

Discussion

Biodiversity of soda lakes is estimated to be lower in comparison to freshwater and marine ecosystems.

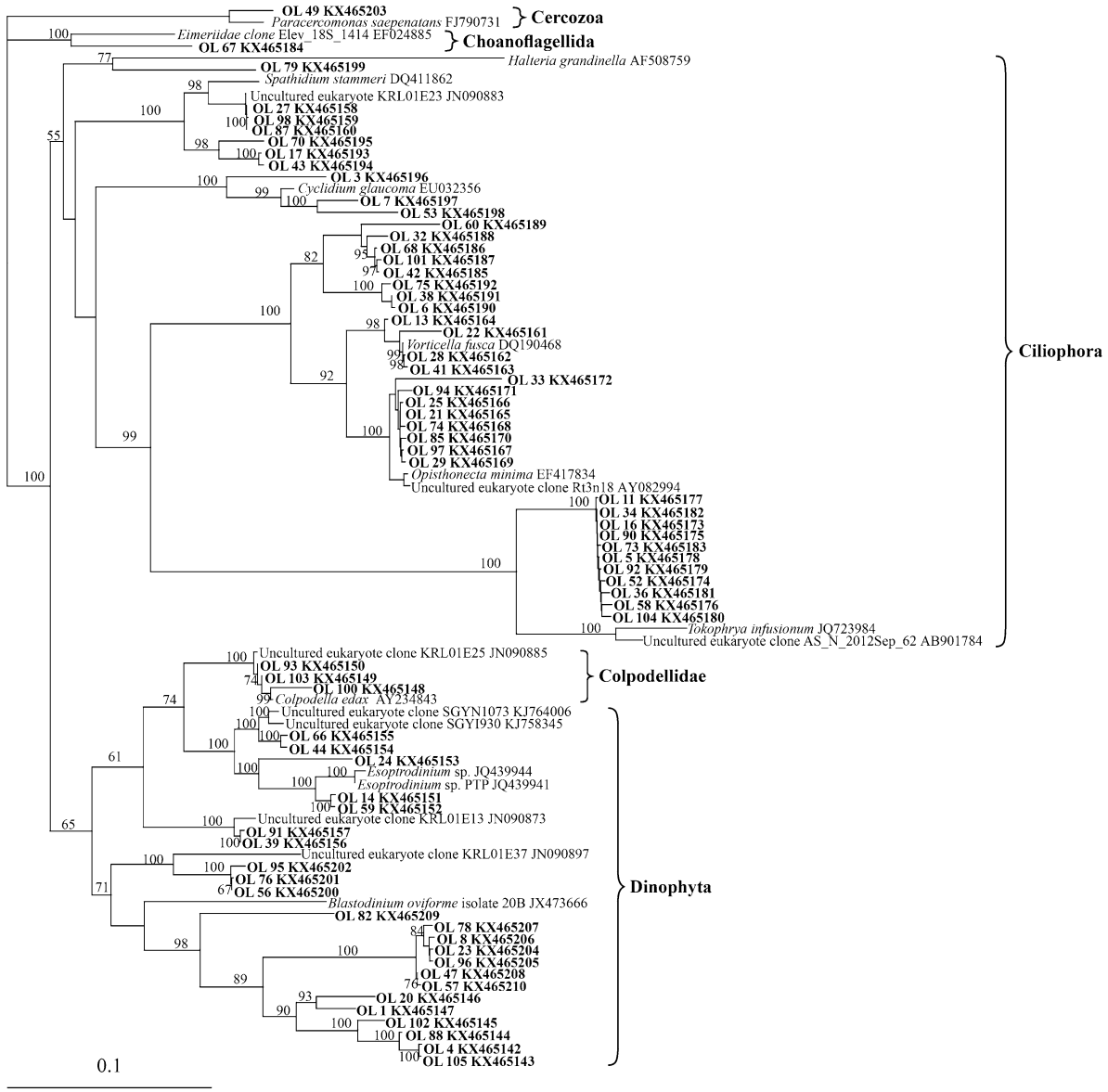


Fig. 3 Phylogenetic relationship of 18S rDNA sequences within the main detected phyla of total libraries found in the sample from 2015: Alveolata. The phylogenetic tree shown was inferred by maximum likelihood method. Bootstrap support values (≥50%) of maximum likelihood (100 replicates) and neighbor-joining analysis (1,000 replicates) are marked in the tree. The *scale bar* indicates the estimated number of base changes per nucleotide sequence position

This situation has been attributed to the harsh living conditions under saline-alkaline milieu (Wood & Talling, 1988; Dudgeon et al., 2006; Costello et al., 2010). A relatively low number of organisms are responsible for the extraordinary high production rates in soda lakes (Krienitz & Schagerl, 2016; Schagerl & Burian, 2016). Some of the highest primary production values reported have been attributed to the principal primary producer *Arthrospira* (Oduor & Schagerl, 2007; Schagerl & Oduor, 2008). The actual biodiversity in saline lakes varies with the lake water salinity level and can be comparatively higher under hyposaline conditions (Schagerl & Burian, 2016) as was the case in Lake Oloidien. A multitude of biogeochemical cycles involving carbon, nitrogen, and sulfur conducted by microbes can lead to an elevated biodiversity in alkaline lakes (Sorokin et al., 2014). Molecular methods revealed that the genetic diversity in soda

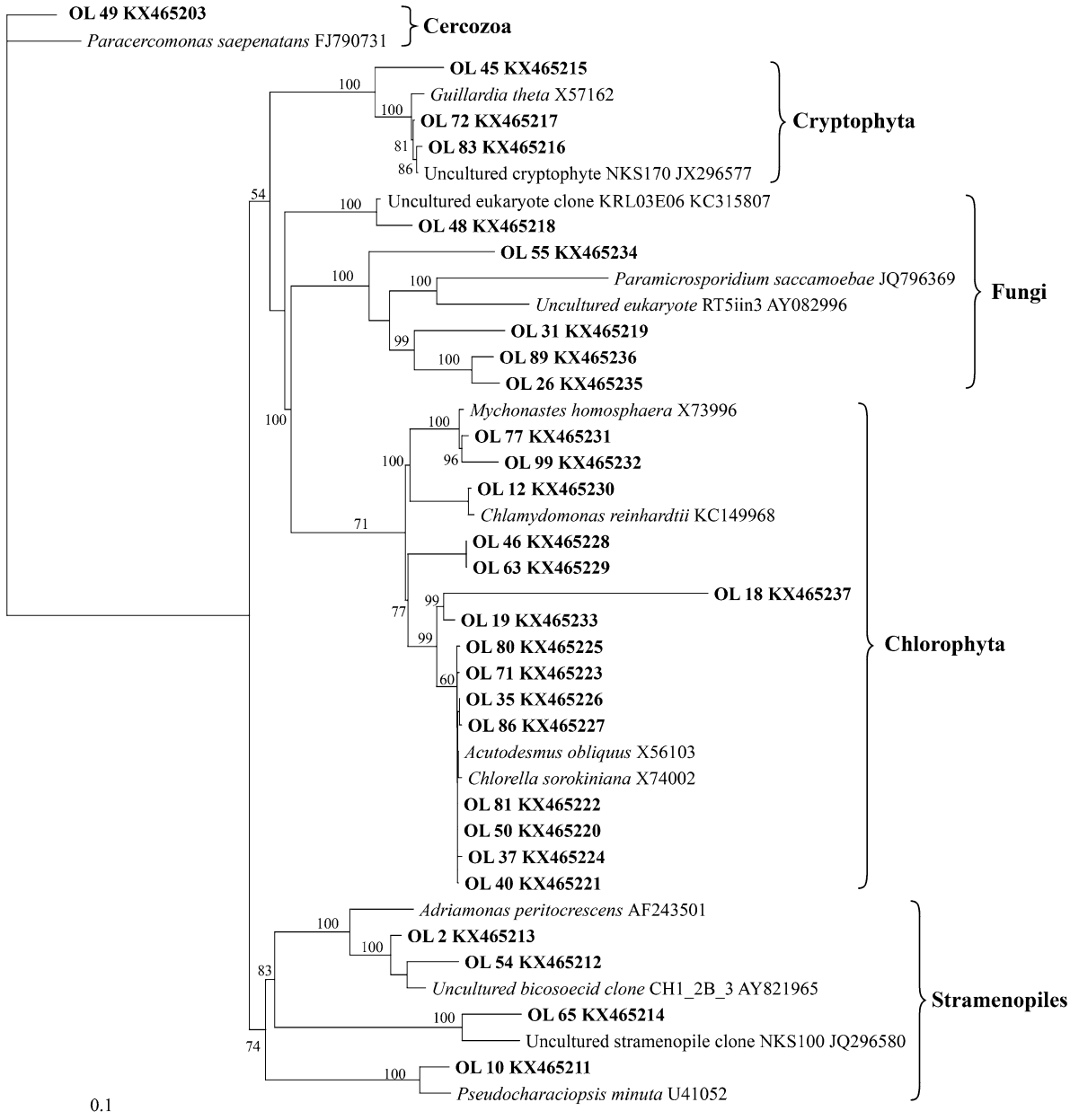


Fig. 4 Phylogenetic relationship of 18S rDNA sequences within the main detected phyla of total libraries found in the sample from 2015: Other microbial Eukaryota. The phylogenetic tree shown was inferred by maximum likelihood method. Bootstrap support values (≥50%) of maximum likelihood (100 replicates) and neighbor-joining analysis (1,000 replicates) are marked in the tree. The *scale bar* indicates the estimated number of base changes per nucleotide sequence position

lakes is comparable to that of other aquatic ecosystems (Lanzén et al., 2013; Luo et al., 2013).

In this paper, we used the opportunity offered by the contrasting water conditions to compare the biodiversity of planktonic organisms in Lake Oloidien in order to evaluate the differences that occurred throughout and after mass developments of the key primary producer *Arthrospira*. It was possible to show remarkable divergences on the level of bacterial and microeukaryotic communities.

In the two samples from Lake Oloidien studied, a high diversity of organisms was detected. However, in most cases, a clear designation at species level was inconclusive or not possible. Many of the genotypes need to be described as new species and their ecological preferences established through ecophysiological experiments. Based on their salinity tolerance, the few clearly designated species belonged to a wide range of fresh and salt water taxa. The interpretation of the community structure of a lake based on salinity should be done with caution owing to the interaction between climate and salinity and the complex response of organisms (as demonstrated in the example of diatom flora of African lakes by Gasse et al., 1997) and involves memory effects in regard to previous stages of the ecosystem (Verschuren, 2003).

Bacteria

During the present study, Firmicutes, Cyanobacteria, Proteobacteria, Bacteroidetes, Chloroflexi, Actinobacteria, Acidobacteria, Verrucomicrobia, Deinococcus–Thermus, Fusobacteria, Gemmatimonadetes, Tenericutes, and others phyla were found in the waters of Lake Oloidien. Previous studies have also traced most of these bacterial phyla in soda lake environments (Rees et al., 2004; Grant & Sorokin, 2011; Borsodi et al., 2013; Lanzén et al., 2013; Sorokin et al., 2014). Moreover, molecular studies of the bacterial communities in soda lake water have also revealed highly diverse bacterial populations dominated by clones affiliated with Firmicutes, Proteobacteria, and Bacteroidetes (Grant & Sorokin, 2011; Grant & Jones, 2016). Bacterial diversity within the hot springs of Lake Magadi and Little Magadi in Kenya were studied by Illumina sequencing (Kambura et al., 2016a). Phyla of Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria, Cyanobacteria, Chloroflexi, and *Deinococcus*–thermus were found dominant in hot spring. The Firmicutes were the most abundant phylum in the spring water at a temperature of 45.1°C of Lake Magadi.

The main difference in the bacterial sequences reads in this study was the dominance of Firmicutes in the library of OL2011, while Cyanobacteria were found to be the most dominant in library OL2015. Furthermore, there was a difference in the dominant class of proteobacteria between OL2011 and OL2015. A higher dominance of Gammaproteobacteria was traced in OL2011 while Betaproteobacteria dominated in OL2015.

The Firmicutes (low-G + C-content gram-positive bacteria) are abundant and easily isolated and were discovered in soda lakes during cultivation (Jones et al., 1998; Borsodi et al., 2008) and molecular surveys (Ma et al., 2004; Rees et al., 2004; Borsodi et al., 2013). The gram-positive bacteria are usually associated with members of the *Bacillus* spectrum. However, the bacterial sequences reads related to Firmicutes were by far the most represented in the OL2011 library. This may have been caused by the diversity of multiple copy numbers of 16S rRNA genes in genomes (Farrelly et al., 1995; Kerkhof & Speck, 1997). Our analyses indicated that OL2011 sample was dominated by low-G + C-content gram-positive bacteria related to *Bacillus*, which mean 16S rRNA gene copy number was 11 after searching in rrnDB (Stoddard et al., 2015). If intragenomic heterogeneity is not the cause of the high diversity in the OL2011 sample, then environmental factors must be contributing to the diversity that was observed. The low-G+C-content gram-positive bacteria were represented by *Bacillus*, *Lactococcus*, and *Enterococcus* (Table 4), facultative anaerobes that are part of the normal gut microflora, and thus might have originated from fecal material produced by the birds (Humayoun et al., 2003).

Gammaproteobacteria were common in samples from OL2011 and OL2015. It was therefore not surprising that the majority of the gram-negative bacterial isolates from the soda lake were members of Gammaproteobacteria (Woese et al., 1985). It is possible that Gammaproteobacteria comprise chemoheterotrophic bacteria, which often makes them the dominant group in the environment. It has been reported that Gammaproteobacteria have ability to grow quickly on a wide range of carbon sources of different concentrations (Uphoff et al., 2001).

The reason for the difference in the dominant class of proteobacteria could be the fluctuation in salt content. Salinity has been reported to be the dominant environmental factor controlling bacterioplankton community composition in high-mountain lakes of Tibet (Wu et al., 2006). The relative abundances of different classes of proteobacteria showed an abrupt switch along the salinity gradient. In general, the relative abundance of Alphaproteobacteria and Gammaproteobacteria increased with increasing

salinity. Betaproteobacteria are a characteristic element of bacteria communities in freshwater habitats (Methé et al., 1998). Meanwhile, the high relative abundance of Betaproteobacteria in freshwater habitats and their decrease with increasing salinity was observed previously in studies on inland waters (Glöckner et al., 1999; Demergasso et al., 2004; Wu et al., 2006).

The Chloroflexi (formerly known as green non-sulfur bacteria) were the second major group in OL2015. This phylum of bacteria contains a high diversity of phenotypes and chemotypes including aerobic thermophiles, anoxygenic phototrophs, and anaerobic halorespirers (Garrity et al., 2002). The Chloroflexi has been divided into four major subdivisions on the basis of 16S rDNA/RNA sequences (Hugenholtz et al., 1998). *Anaerolinea* and *Caldilinea*, which belong to subdivision I, were the most detected members of Chloroflexi from sample OL2015. The reads grouped into *Anaerolinea* and *Caldilinea* were closely related to uncultured Chloroflexi clones (Table 5). The type strains *Anaerolinea thermophile* Sekiguchi et al. UNI-1^{T} and *Caldilinea aerophila* Sekiguchi et al. STL-6-O1^{T} (Sekiguchi et al., 2003) are thermophilic, Gram-negative, non-spore-forming, multicellular filamentous, chemo-organotrophic bacterial isolates.

Cyanobacteria

For the cyanobacteria, it was possible to compare the findings of light microscopy with molecular studies. The general outcomes were covered by both approaches. Only one dominant (*Arthrospira*), and two subdominant taxa (*Anabaenopsis*, *Microcystis*) were traced in OL2011. The *Arthrospira fusiformis* population was established by a small-celled ecotype of the species (Krienitz et al., 2013a) which is genetically similar to larger celled strains (Dadheech et al., 2010). *Anabaenopsis* was represented by *A. elenkinii* (Krienitz et al., 2013b), and *Microcystis* by *M. aeruginosa* (Krienitz et al., 2013c). In OL 2015, *Arthrospira* was absent, and instead 13 genera were detected by microscopy and 10 genera by molecular methods. The lower number of genera obtained through molecular detections possibly resulted from the difficulties associated with the adjustment of sequences of picoplanktonic taxa to known species. Normally, picoplanktonic cyanobacteria are solitary; however, colonies containing cells of picoplankton size can disintegrate to individual cells and mimic solitary taxa. Many of such taxa were misinterpreted as *Synechococcus*, a solitary or pseudofilamentous genus suspected to be widely distributed in all aquatic ecosystems (Callieri et al., 2012, 2013). Dvořák et al. (2014) pointed to an inflation of *Synechococcus* lines, and demonstrated the existence of "extremely polyphyletic relationships in *Synechococcus*, which has not been observed in other cyanobacteria" (loc. cit p. 5538). A clone library collected from the saline-alkaline lake Elmentaita in the African Rift Valley contained 96 clones of cyanobacteria, 75 of them belonged to *Synechococcus* and only 21 were grouped to nine other genera (Mwirichia et al., 2011). A notable observation was the identification by molecular methods of *Cyanobacterium* sp. and *Geminocystis* sp. in sample OL2015 which though microscopic work could be confused with "*Synechococcus*." According to Komárek & Anagnostidis (1999) until now, seven species of *Cyanobacterium* have been delineated from very different habitats that range from freshwater to saline or thermal places. Other species are awaiting taxonomic treatment. Korelusová et al. (2009) separated the new genus *Geminocystis* from *Synechocystis* and established the new genus as sister lineage to *Cyanobacterium*. Microscopic examination revealed that *Raphidiopsis curvata* was one of the dominant cyanobacteria in OL2015. However, the species could not be detected by molecular methods. This is possibly because this taxon is yet to be deposited in the GenBank or it is hidden under an uncultured cyanobacterium clone.

Microbial eukaryotes

Metazoan sequences reads (in concordance with microscopic detection of *Brachionus dimidiatus*) were dominant in OL2011 while the microbial eukaryotes, especially Ciliophora within Alveolata were often traced. According to Burian et al. (2013) and Mengistou (2016) *B. dimidiatus* is common in Rift Valley lakes, however, its grazing impact on intact filaments of *Arthrospira* is relatively low. *B. dimidiatus* preferably ingests fragmented or decomposed cyanobacteria, small flagellates and algae and Bacteria. Microalgae such as Cryptophyta (mostly *Guillardia*) were the main phototrophic eukaryotic phytoplankton. In OL2015, higher microbial eukaryotes diversity was confirmed

by both molecular protocols; Illumina Miseq sequencing and 18S rRNA clone library. Ciliophora, Cryptophyta, and Chlorophyta were the most important clades during this period.

Alveolates were mainly represented by Ciliophora, the bacterivorous and herbivorous consumers. Because of the high concentration of bacterial and microphytan food organisms in the plankton, the abundance and production of ciliated protozoans is higher in soda lakes of East Africa than in freshwaters and lakes of the temperate zone (Yasindi & Tailor, 2016). A higher abundance of ciliates recorded in 2011 confirmed this conclusion. However, the information currently available is inadequate to demonstrate a correlation between ciliate abundance and abiotic and biotic factors (Macek et al., 2006; Ong'ondo et al., 2013).

Comparing the list of eight genera revealed by molecular investigation with the findings of a detailed microscopic analysis of ciliates in the study region (Yasindi et al., 2007), a close similarity between the dominant taxa was established. However, new elements of ciliate fauna were revealed hence the need for further research (see also the high number of unnamed OTUs in Fig. 3). Yasindi et al. (2007) studied Lake Oloidien at conductivity stage of 2,466 μS cm^{-1} which was very similar to the conditions measured in our OL2015 sample with *Cyclidium* and *Vorticella* being the dominant cilliates. *Vorticella* is a voracious grazer of picocyanobacteria supported by the rich occurrence of *Synechococcus* in Oloidien 2015. So far 39 genera of ciliates have been recorded in the soda lakes of East Africa (Ong'ondo et al., 2013). Based on morphological and molecular (SSU rRNA gene) analyses, Ong'ondo et al. (2013) proposed that most of the taxa found should be described as new species. Five of the genera traced by our analyses are new to the Rift Valley soda lakes: *Ichthyophthirius*, *Linostomella*, *Opistonecta*, *Peritrichia*, and *Tokophrya*. According to Matthews (2005) and MacColl et al. (2015) *Ichthyophthirius multifiliis* Fouquet is a pathogen of fishes (Ichthyophthiriosis).

According to Zinabu & Taylor (1997), high densities of ciliates reduce numbers of heterotrophic nanoflagellates. In the present study, we only identified *Paracercomonas saepenatans*, a little known cercomonad described from farmland-soil in UK from this group (Bass et al., 2009). Nevertheless, other flagellates such as *Colpodella edax* (Klebs) Simpson et Patterson were found acting as predators. These flagellates are known from ponds, hunting and digesting free-living protists by myzocytosis (Leander et al., 2003). The Colpodellidae are a sister group to Dinophyta (dinoflagellates). The dinoflagellates are a very important ecological group in the aquatic environments with a wide range of nutritional strategies from heterotroph, mixotroph to phototroph with some of them acting as strong predators equipped with an array of strategies for capturing prey (Schnepf & Elbrächter, 1992). Unfortunately, in soda lakes of the African Rift Valley no detailed studies on dinoflagellates have been conducted. Beside the numerous unknown OTUs recorded, we found *Blastodinium oviforme* Chatton, a parasite of marine copepods (Skovgaard & Saiz, 2006), and members of the mixotrophic (parasitic and phototrophic) *Esoptrodinium* that live in freshwaters (Fawcett & Parrow, 2014).

The Stramenopiles were represented by few findings from different algal groups. The pennate diatom *Anomoeoneis sphaerophora* Pfitzer is common in littoral zones of different habitats, from limnic to brackish water and also inland salt lakes (Krammer & Lange-Bertalot, 1986). The picocyanobacteria eating chrysophyte flagellate *Poterioochromonas malhamensis* (Pringsheim) Peterfi was shown to be toxic to *Brachionus* (Boxhorn et al., 1998). Bicosoecid flagellates were also found (*Adriamonas* sp., and an unnamed clone); however, little is known about their function in the ecosystem. The facultative pathogen *Blastocystis* was found in OL2011. Its origin was suspected to be the digestive tract of the flamingos.

The cryptomonade *Gullardia theta*, which was dominant in OL2015 was recorded for the first time in the African soda lake Nakuru by Luo et al. (2013) and is originally known from coastal waters of North America (Hill & Wetherbee, 1990).

In 2015, a highly diverse community of green algae was detected in Lake Oloidien by both microscopic and molecular approaches. Members of the genera *Chlamydomonas*, *Acutodesmus*, *Chlorella*, *Mychonastes*, and *Scenedesmus* were the most frequently encountered. In the determination of the taxa present, the two methods complemented each other. Morphological uniform taxa such as *Mychonastes* and *Chlorella* were better identified by molecular tools in addition to microscopy. The relatives of *Chlorella*, *Mychonastes*, and *Scenedesmus* are so close together, that only few distinctions in sequences can discriminate between

different species (Hegewald et al., 2010; Luo et al., 2010). Consequently, under a "similarity" of 99% a wide diversity of different taxa can be hidden, and the potential for discovering of new taxa from the African continent is very high as shown by Krienitz et al. (2011, 2012). A study by Luo et al. (2013) revealed a high number of chlorophyte genotypes in Lake Nakuru in the phase of declining salinity. Out of 77 clones of eukaryotic plankton recorded, 52 belonged to Chlorophyta.

Fungi

In general, information on the diversity of aquatic fungi is limited, especially in soda lake ecosystems. However, their unique metabolic ability makes them the drivers of remineralisation of organic matter and energy cycling in aquatic food webs (Grossart & Rojas-Jimenez, 2016). Comparatively, the species composition of fungi in soda lakes is far less than that of other inland waters. Luo et al. (2013) provided first evidence of the presence of Chytridiomycota in Lake Nakuru. A study by Kambura et al. (2016b) provides the first report on the fungal richness of sediments and water of hot springs of Lake Magadi. Using molecular tools, the authors detected many "transition" taxa known from other waters with very distinct environmental conditions from those of saline-alkaline lakes. For example, an unnamed taxon from Antarctica was discovered. We detected *Paramicrosporidium,* which has been recorded in the Antarctic snow (unpubl. sequence in GenBank, however with a low similarity of 83%). Altogether, we found members of six genera of fungi and several unnamed clones. The abundance of fungi was higher in OL2011 than in OL2015.

Comparison with other soda lakes in the East African Rift Valley

Among the five Ethiopian soda lakes investigated by Lanzén et al. (2013), the crater lake Aranguadi (salinity 2.8 ppt) showed the highest similarity to Lake Oloidien in terms of genesis, morphometry, physical and chemical characteristics as well as dominance by the small-celled ecotype of *Arthrospira fusiformis* in the phytoplankton (Girma et al., 2012). The microbial clades found by Lanzén et al. (2013) were a mixture of OTUs that occurred in Oloidien in the two different stages.

In Lake Oloidien, although a small difference in the salinity level of the two samples was recorded (2.6 ppt), a wide difference in organism composition was observed. In the same period, a pronounced decline in the salinity of the soda lakes of the Great African Rift Valley resulting from a large volume of freshwater inflow, was observed between 2011 and 2015. The main flamingo lakes in the region experienced a decline of 12.7 ppt (Lake Bogoria) and 7.6 ppt (Lake Nakuru) (Krienitz et al., 2016a). In all of these flamingo lakes, a critical concentration of salt fell below the value which supports a high performance of the cyanobacterium *Arthrospira*. Although the magnitude of salinity changes in each of the lakes was different, the impact on the flamingo food chain was similar—a total breakdown.

In 2011, the lake conditions in Oloidien were conducive for massive blooms of *Arthrospira* that attracted tens of thousands Lesser Flamingos which contributed to a bacterial flora dominated by Firmicutes, especially Bacilli originating from the high volume of fecal waste from the birds. A decline in lake water salinity in 2015 led to the disappearance of *Arthrospira* and the flamingos from the lake, and the dominance of the bacterioplankton by an elevated number of autotrophic cyanobacteria. Furthermore, a high diversity of algae and flagellates prevailed in this phase. Our results documented the excursiveness and hidden diversity of soda lakes ecosystems.

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