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Bacterial and archaeal communities in Lake Nyos (Cameroon, Central Africa)

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The aim of this study was to assess the microbial diversity associated with Lake Nyos, a lake with an unusual chemistry in Cameroon. Water samples were collected during the dry season on March 2013. Bacterial and archaeal communities were profiled using Polymerase Chain Reaction-Denaturing Gradient Gel Electrophoresis (PCR-DGGE) approach of the 16S rRNA gene. The results indicate a stratification of both communities along the water column. Altogether, the physico-chemical data and microbial sequences suggest a close correspondence of the potential microbial functions to the physico-chemical pattern of the lake. We also obtained evidence of a rich microbial diversity likely to include several novel microorganisms of environmental importance in the large unexplored microbial reservoir of Lake Nyos.

Microorganisms constitute a substantial proportion of the biosphere. Their number is at least two to three orders of magnitude larger than that of all the plant and animal cells combined, constituting about 60% of the earth's biomass[1]; besides, they are very diverse. This significant representation allows them to stand as controllers of the habitability of the planet through the key roles they play in biogeochemical cycles and food webs[2,3]. Amongst them are 3 very important groups that evolved from the same ancestor, archaea, bacteria and eukarya. On a genetic basis, they belong to the so called "three-domain system"[4]. They are at the base of the foodwebs in numerous environments where their several metabolisms mobilise the energy[5]. Over the past decades, researchers have used these 3 groups of microorganisms to study the aquatic systems. The methods used in microbial ecology progressed from standard culture techniques to modern molecular biology methods. These molecular biological methods are based on the 16S (18S) rRNA genes and revealed that several candidate divisions could not be studied using traditional culture techniques[6]. This remark makes the molecular techniques in microbial ecology crucial for an intensive characterisation of microbial communities in a given environment. DGGE is one of the numerous fingerprinting approaches that have been designed to study microbial communities, what culture techniques would not allow given that the cultivable fraction represents $<1\%$ of the total number of prokaryotic species present in a given sample[6]. DGGE was used in this study to explore the microbial diversity in Lake Nyos for the first time.

Lake Nyos (Figure 1) is 210 m deep and permanently stratified[7,8]. Its water column is divided into layers separated by vertical gradients of temperature and dissolved chemical species. The CO_2 from deep-magmatic origin reaches the bottom and dissolves into the lake water[7,8,9]. In 1986, the lake suddenly released a cloud of carbon dioxide into the atmosphere, killing about 1,800 people and 3,000 livestock in nearby towns and villages. This devastating event led to the "Killer Lake" appellation. Since the catastrophe, several studies led to a better understanding of the lake's geological and physico-chemical characteristics. Notably, the CO_2 content of the lake increases towards the bottom (the anoxic environment is produced primarily by the absence of dissolved oxygen). Small amounts of endogenic siderite and traces of pyrite have been found in the sediments[10]. The dissolved chemical species are overwhelmingly dominated by CO_2 and HCO_3^-, followed by Fe^{2+}. Apart from Lake Nyos, two other lakes are known to be stratified and accumulate gas: 1) Lake Monoun in Cameroon and 2) Lake Kivu in the Rwanda-Democratic Republic of Congo border. Among the numerous meromictic lakes worldwide, those three lakes are unique in terms of the amount of gas in the hypolimnion[11]. In the aftermath of the gas explosion at Lake Nyos, the works of Kling *et al.*[12] and Kusakabe *et al.*[8] showed that the gas started to accumulate again; to avert recurrence of catastrophe, it was suggested to degas the lake. Degassing is done since 2001 and the physico-chemical parameters are continuously monitored[12,8].

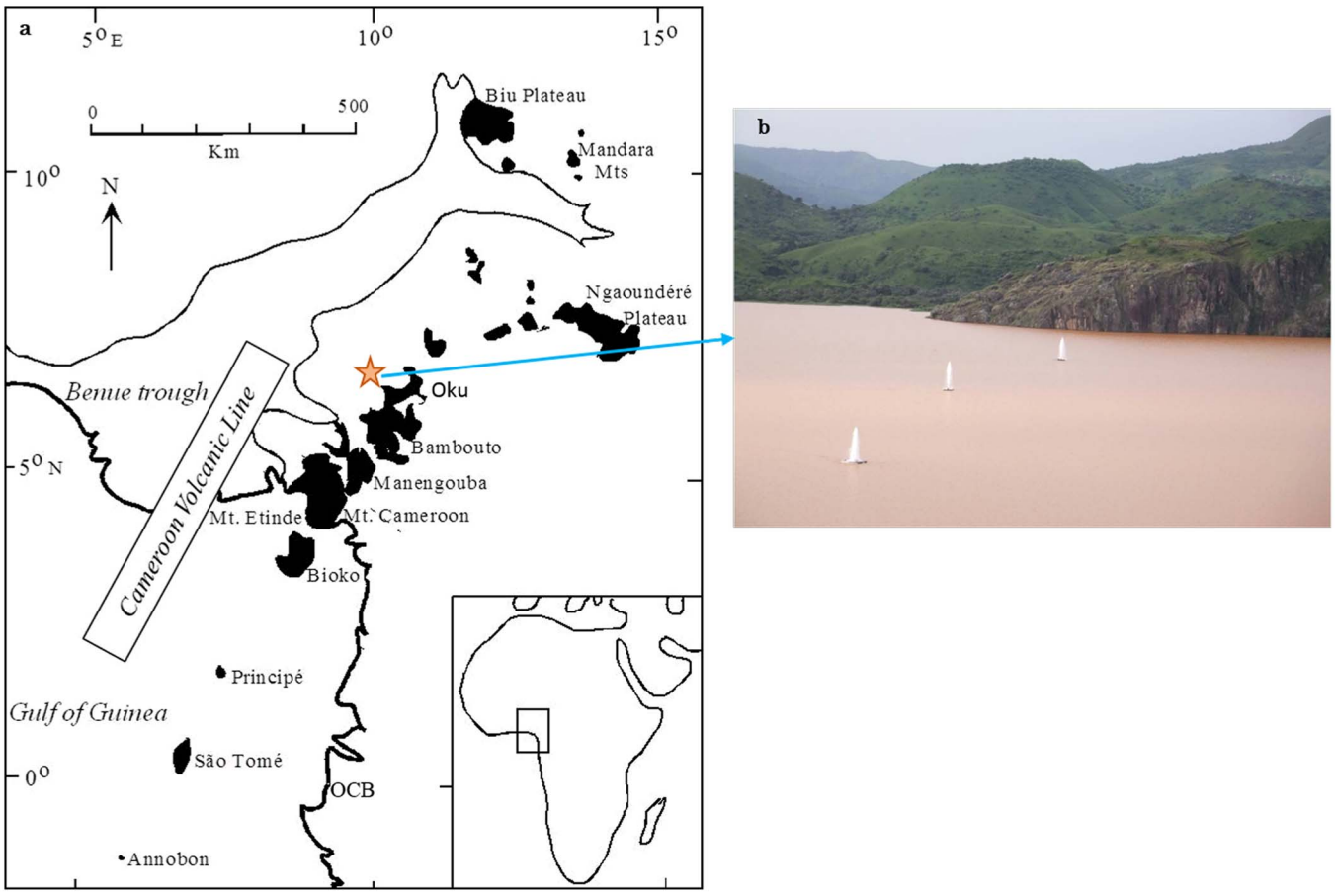

Figure 1 | Geographical location of Lake Nyos (a) and View of the lake in March 2013 (b). OCB: Ocean Continent Boundary; Map: the map was modified from Aka[60]; Lake's picture: Photo courtesy of Yutaka Yoshida.

Despite the important role that microorganisms play in the geochemical processes, no research has been conducted on the biology of the lake; for that reason, we focused here on the determination of bacterial and archaeal communities, and on the understanding of their role in the geochemical processes of the lake.

Results

Physico-chemical characteristics of the samples. Plots of the physico-chemical parameters (Table S1) of the lake in March 2013 are presented in Figure 2 which shows the overwhelming domination of $CO_{2(aq)}$ (a), HCO_3^- (b) and Fe^{2+} (c) species. The CO_2 concentrations (Figure 2a) increased from 0.2 to 153 mmol kg^{-1}. Similar to the conductivity profile, bicarbonates increased from 2.4 to 38.9 mmol kg^{-1} with a slight decrease (2 to 1.1 mmol kg^{-1}) from −45 to −80 m (Figure 2b). The Fe^{2+} concentration was low from 0 to −80 m, then increased considerably from −90 to −210 m (Figure 2c). The other chemical species followed the same trend (increasing but not abruptly as Fe^{2+}) with exception of SO_4^{2-} and NO_3^- (Figure 2d), which decreased from the surface to the bottom. The acidity slightly decreased while the temperature slightly increased from about −70 m to the bottom of the lake. Dissolved oxygen (DO) was not detected at the depths where it was measured (−100, −120, −140, −200 and −210 m).

DGGE profiles of microbial community structures based on 16S rRNA and ecological indexes. The number of detectable bands varied from 6 to 23 per track for bacteria (Figure 3a); for archaea (Figure 4a), fewer bands were observed and varied from 2 to 15 per track. The similarities of all gel tracks were calculated to determine the information content of the banding patterns in terms of the structural diversity of the samples. A cluster analysis of the matrix of similarity values was then performed and visualised in a dendrogram for bacteria (Figure 3b) and archaea (Figure 4b).

The bacterial banding pattern was differentiated into four clusters as a function of depth as follows: two upper clusters from 0 to −10 m, −25 to −80 m and two lower clusters from −90 to −160 m and −180 to −210 m. The archaeal banding pattern showed 3 clusters: ranging from −25 to −80 m, −90 to −180 m and −200 to −210 m.

PCA plot performed on the physico-chemical variables per sampling depth (Figure 5) shows the clustering between sampling sites and physico-chemical parameters. The first PC (PC1) displayed a greater variation of the sampling sites and a negative correlation with the physico-chemical parameters, while the second PC (PC2) showed a positive correlation with the parameters. While the majority of the parameters had a very close orientation pattern, SO_4^{2-} and pH seemed to have a different orientation. The parameters seemed to have more relation with the deepest depths (from −100 to −210 m). MDS plots produced a similar clustering pattern as the dendrograms, showing four clusters for bacteria (Figure 6a) and three for archaea (Figure 6b).

Bacterial and archaeal community structure and distribution. A total of 46 bands were successfully sequenced for bacteria and 15 bands for archaea. Bacteria showed a higher diversity (Figure 3c) than archaea (Figure 4c), with a DGGE profile that was far more complex. The successfully sequenced bands are indicated with arabic numerals on the DGGE gels (Figures 3a and 4a). The obtained sequences were aligned to the GenBank sequences of the 16S rRNA gene for bacteria and archaea database; the

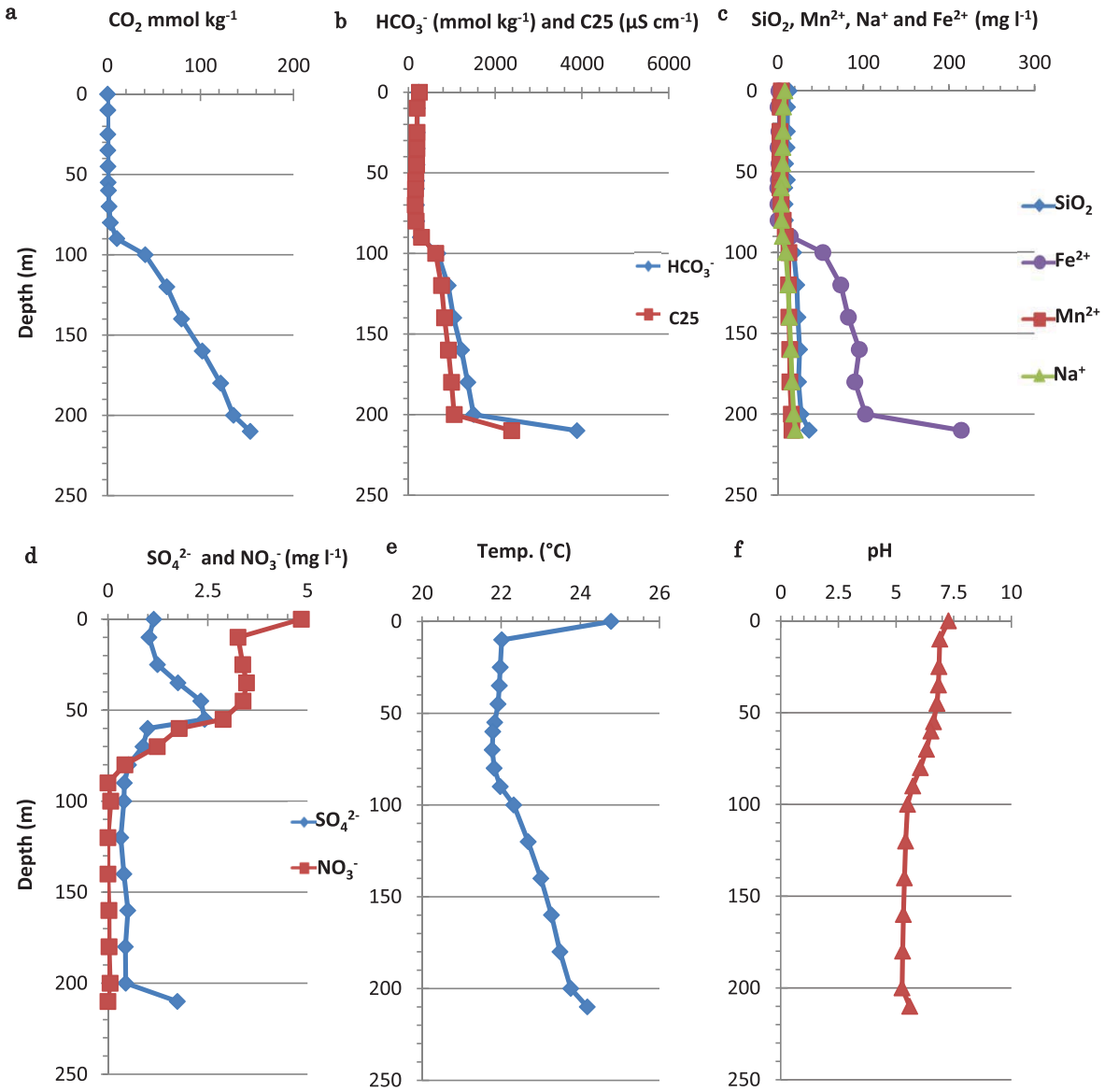

Figure 2 | Water depth profiles: carbone dioxide (CO_2) (a); bicarbonates (HCO_3^-) (bicarbonate values have been multiplied by 100) and conductivity (C25) (b); silica (SiO_2), manganese (Mn^{2+}) (manganese values have been multiplied by 10), sodium (Na^+) and ferrous iron (Fe^{2+})(c); sulfate (SO_4^{2-}) (sulfate values have been multiplied by 10) and nitrate (NO^{3-}) (d); temperature (T) (e) and hydrogen potential (pH) (f).

nearest relatives were identified for bacterial (Table 1) and archaeal sequences (Table 2). All the sequences that were ≥97% similar to the same organism were grouped under the same genotype and named as Nyos bacteria (Table 1) or Nyos archaea (Table 2) genotypes. Both bacteria and archaea domains exhibited distinct communities at different depths of the lake's water column and their dendrograms indicated a stratified pattern along the water column (Figures 3b and 4b) with the largest difference occuring around 80–90 m depth. The bulk of the matches of bacterial sequences belonged to the phyla Firmicutes and Actinobacteria that accounted each for about 28.3%. They dominated from 0 to −55 m, while fewer representatives were found at other depths. However, when downscaling, some differences arose to finer taxonomic resolution. The matches of the detected sequences belonged to the phyla Proteobacteria (21.7%), Bacteroidetes (8.7%), Caldiserica (4.4%), and 2.2% for each of the phyla Ignavibacteriae, Nitrospirae, Tenericutes and Fusobacteria. For the archaeal sequences, the closest relatives belonged to the Thaumacheota (53.3%), Euryarchaeota (33.3%) and Crenarcheota (13.3%).

Relationship between bacterial and archaeal communities with the various depths. The vertical profiles of the physicochemical parameters of the lake showed a stratification with a considerable increase of concentrations starting from about −80 m for CO_2, HCO_3^-, Fe^{2+} and C25. Mn^{2+}, Na^+ and SiO_2 concentrations slightly decreased around −60 m and increased again around −100 m. NO_3^- and SO_4^{2-} concentrations rather kept a reducing trend along the column towards the bottom and almost stabilised at −80 m. Na^+ concentration was very low in the upper part of the lake and started increasing gradually around −80 m to reach its maximum concentrations towards the bottom. These chemical features could map with the identified bacterial and archaeal sequences, suggesting a close relationship between the potential functions of the bacterial/archaeal communities and the physico-chemical characteristics of the various layers of the lake. Even though the archaeal community was less diverse, its stratification pattern was clearly displayed.

The bacterial bands obtained from 0 to −35 m were highly similar to the aerobic *Exiguobacterium* sp., and *Arthrobacter* sp.; sequences close to *Micropruirina glycogenica*, a facultative anaerobic bacterium

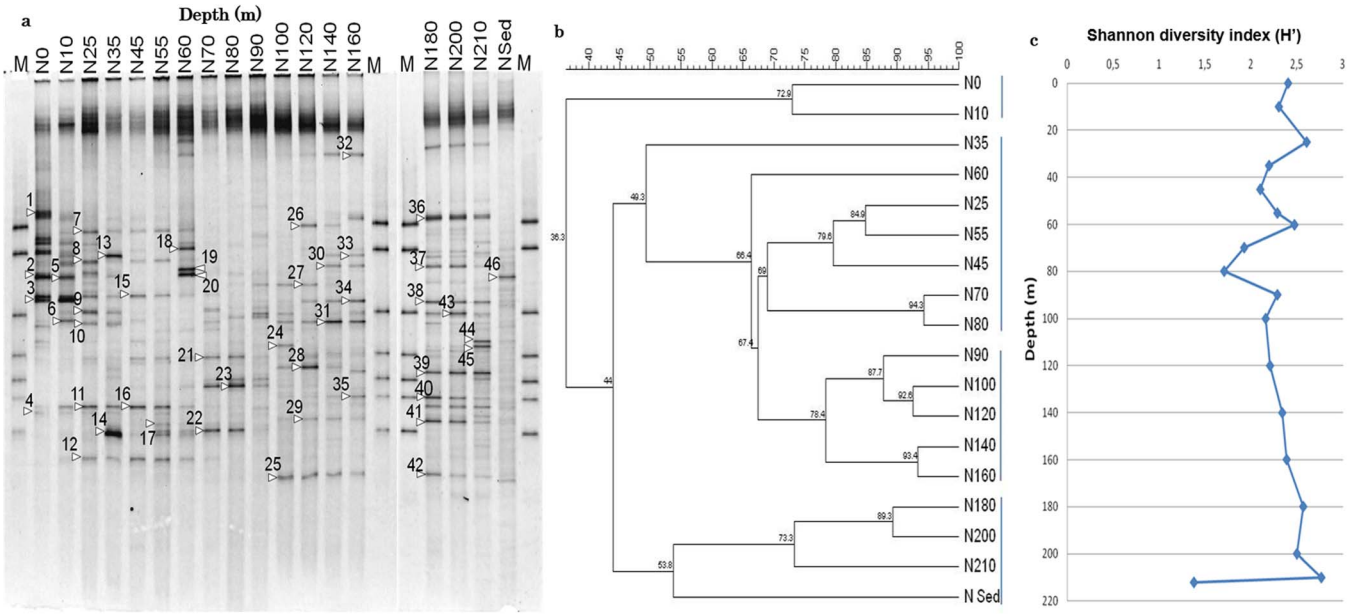

Figure 3 | Denaturing gradient gel electrophoresis (DGGE) profiles of bacterial communities at different depths in Lake Nyos (a). Dendrogram calculated with the clustering algorithm of Unweighted Pair-Group Method with an Arithmetic Mean (UPGMA) for bacteria in samples from all depths (b). Vertical changes of Shannon-Weaver index of diversity (H') based on the number and relative intensities of the bands for bacteria identified by DGGE analysis of PCR-amplified 16S rRNA gene (c). **NSed:** Sediment sample collected at the bottom of the lake. **Arabic numerals:** Successfully sequenced bands for bacteria. **M:** Mass ladder standards.

and capable to reduce nitrate as mentioned by Shintani *et al.*[13] were also present. Organisms close to the *Acidimicrobium ferrooxidans*, capable of ferrous iron oxidation and carbon dioxide fixation[14] were found at −45, −120, −160 and −180 m. Moreover, a sequence close to *Ferrithrix thermotolerans* strain Y005 was detected at −45 m; the latter is able to reduce and oxidise iron; furthermore, it is capable of oxidative dissolution of pyrite[15]. *Melioribacter roseus* strain P3M may also be able to use ferric iron as electron acceptor as mentioned by Kadnikov *et al.*[16] and corresponded to the closest match of one of the bands from the −120 m sample. A sequence related to *Methylocystis echinoides* strain IMET 10491 was found at −70m; the latter is an anaerobic organism capable of methane-oxidation[17]. Sequences matching with the thiosulfate oxidising and carbon dioxide fixing bacteria *Thiobacillus thiophilus* strain D24TN[18] were found at −80 m. Sequences of the genotype 26 that appeared among the bands generated from the sample of −180 m could also play a part

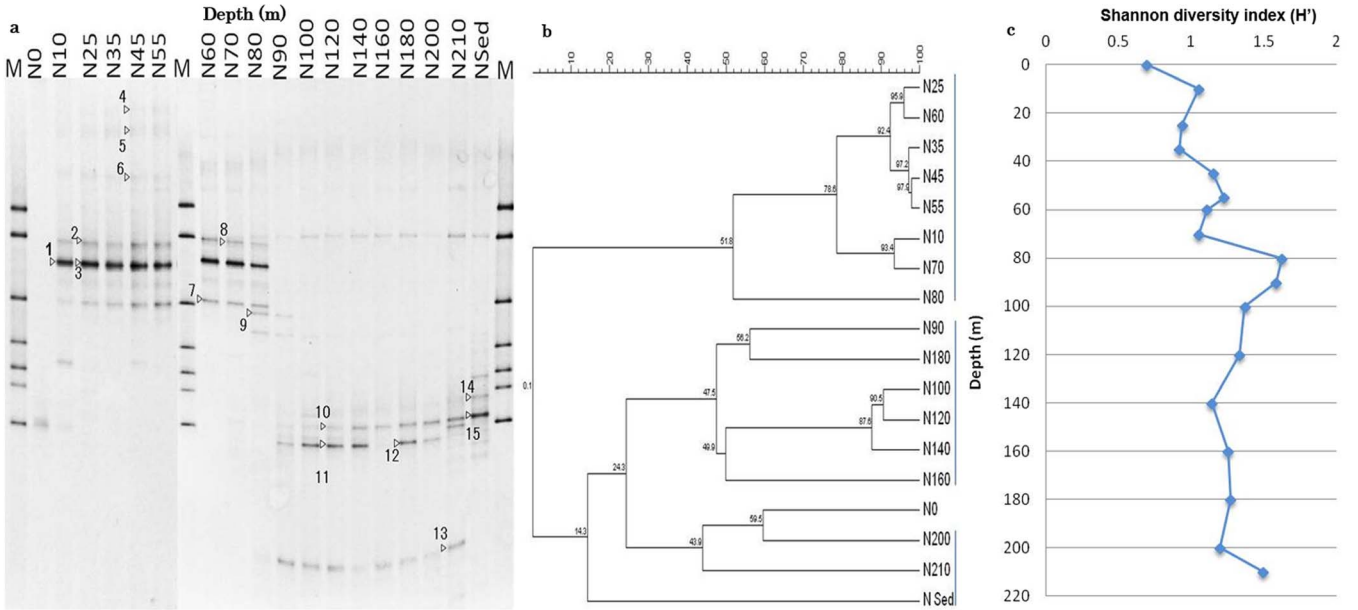

Figure 4 | Denaturing gradient gel electrophoresis (DGGE) profiles of archaeal communities at different depths in Lake Nyos (a). Dendrogram calculated with the clustering algorithm of Unweighted Pair-Group Method with an Arithmetic Mean (UPGMA) for archaea in samples from all depths (b). Vertical changes of Shannon-Weaver index of diversity (H') based on the number and relative intensities of the bands for archaea identified by DGGE analysis of PCR-amplified 16S rRNA gene (c). **NSed:** Sediment sample collected at the bottom of the lake. **Arabic numerals:** Successfully sequenced bands for archaea. **M:** Mass ladder standards.

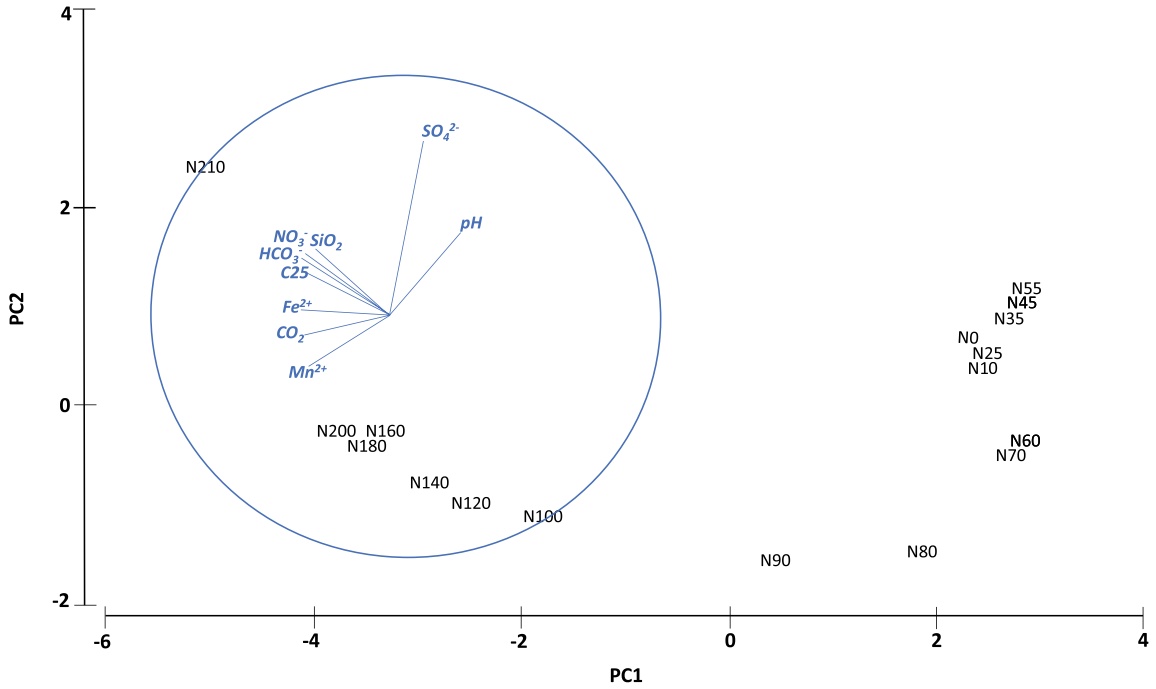

Figure 5 | Principal component analysis (PCA) of Lake Nyos showing the depth-related differences and the structuring role of the physico-chemical parameters. CO_2: Carbon Dioxide; HCO_3^-: Bicarbonates; Fe^{2+}: Ferrous Iron; C25: Conductivity; Mn^{2+}: Manganese; Na^+: Sodium; SiO_2: Silica; pH: Hydrogen Potential; NO_3^-: Nitrate and SO_4^{2-}: Sulfate.

in the thiosulfate metabolism as they matched with *Caldisericum exile* strain AZM16c01^T isolated from a hot spring in Japan and reported as thiosulfate-reducing bacterium[19]. Sequences close to the bacteria (*Desulfovibrio vulgaris* strain DP4 (−160 m), *Desulfotomaculum australicum* strain AB33 (−100), *Desulfovibrio alaskensis* strain G20 (−160 and −180 m)) that contribute to sulfate metabolism as reported by Zane *et al.*[20], Love *et al.*[21] and Hauser *et al.*[22], respectively, and the bacteria (Candidatus *Nitrospira defluvii* (−180 m), *Bacillus alkalinitrilicus* strain ANL-iso4 (−140 m), *Melioribacter roseus* strain P3M (−120 m)) involved in the nitrogen metabolism as mentioned by Lücker *et al.*[23], Sorokin *et al.*[24] and Podosokorskaya *et al.*[25], respectively were detected. One of the bands sequenced at −140 m was the close relative of *Clostridium celluloly-ticum* H10, reported to produce H_2[26]. Another putative hydrogen

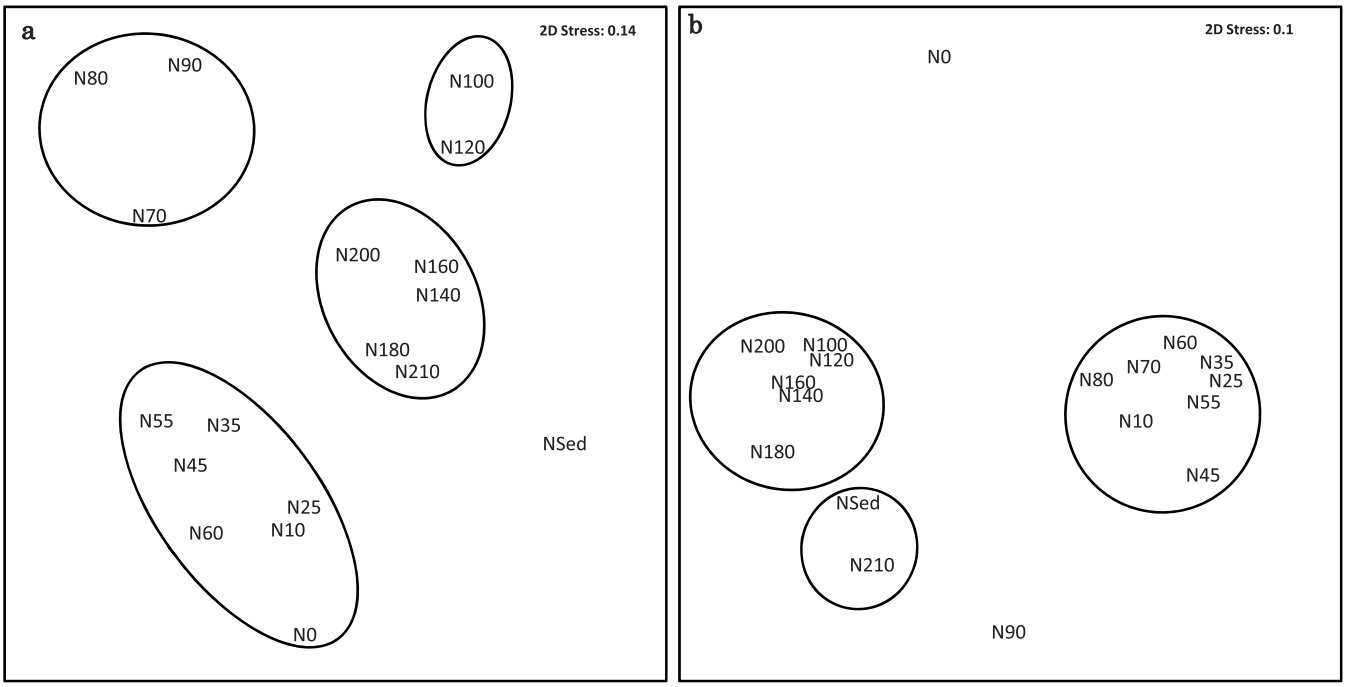

Figure 6 | Multidimensional scaling plots produced to compare the sampling depths for bacteria (a) and archaea (b). NSed: Sediment sample collected at the bottom of the lake.

Table 1 | Bacterial sequences identified at different depths of Lake Nyos and their closest matches to the 16S rRNA gene sequences for bacteria database in the GenBank

		Band				Closest match	
No	Depth (m)	Phylum	Genotype[a,b]	Accession no.	Similarity	Organism	Accession no.
1	0	Firmicutes	NyosB1 genotype 1_1	AB907636	100%	*Exiguobacterium undae* strain DSM 14481	NR_043477
2	0	Firmicutes	NyosB2 genotype 1_2	AB907637	100%	*Exiguobacterium undae* strain DSM 14481	NR_043477
3	0	Firmicutes	NyosB3 genotype 1_3	AB907638	100%	*Exiguobacterium undae* strain DSM 14481	NR_043477
4	0	Actinobacteria	NyosB4 genotype 2	AB907639	97%	*Solirubrobacter soli* strain Gsoil 355	NR_041365
5	10	Firmicutes	NyosB5 genotype 1_4	AB907640	100%	*Exiguobacterium undae* strain DSM 14481	NR_043477
6	10	Firmicutes	NyosB6 genotype 1_5	AB907641	99%	*Exiguobacterium undae* strain DSM 14481	NR_043477
7	25	Actinobacteria	NyosB7 genotype 3_1	AB907642	93%	*Micropruina glycogenica* strain Lg2	NR_024676
8	25	Firmicutes	NyosB8 genotype 4_1	AB907643	99%	*Exiguobacterium acetylicum* strain DSM 20416	NR_043479
9	25	Firmicutes	NyosB9 genotype 4_2	AB907644	97%	*Exiguobacterium acetylicum* strain DSM 20416	NR_043479
10	25	Firmicutes	NyosB10 genotype 5	AB907645	100%	*Exiguobacterium indicum* strain HHS 31	NR_042347
11	25	Actinobacteria	NyosB11 genotype 6	AB907646	95%	*Solirubrobacter soli* strain Gsoil 355	NR_041365
12	25	Actinobacteria	NyosB12 genotype 3_2	AB907647	93%	*Micropruina glycogenica* strain Lg2	NR_024676
13	35	Gammaproteobacteria	NyosB13 genotype 7	AB907648	100%	*Acinetobacter radioresistens* strain FO-1	NR_026210
14	35	Actinobacteria	NyosB14 genotype 8	AB907649	100%	*Arthrobacter phenanthrenivorans* Sphe3	NR_074770
15	45	Actinobacteria	NyosB15 genotype 9	AB907650	93%	*Arthrobacter phenanthrenivorans* Sphe3	NR_042469
16	45	Actinobacteria	NyosB16 genotype 10_1	AB907651	92%	*Acidimicrobium ferrooxidans* strain DSM 10331	NR_074390
17	55	Actinobacteria	NyosB17 genotype 11	AB907652	94%	*Ferrithrix thermotolerans* strain Y005	NR_042751
18	60	Gammaproteobacteria	NyosB18 genotype 12	AB907653	99%	*Pseudomonas brassicacearum subsp. brassicacearum NFM421*	NR_074834
19	60	Betaproteobacteria	NyosB19 genotype 13	AB907654	99%	*Janthinobacterium lividum* strain DSM 1522	NR_026365
20	60	Betaproteobacteria	NyosB20 genotype 14	AB907655	95%	*Collimonas fungivorans* Ter331	NR_074756
21	70	Actinobacteria	NyosB21 genotype 15	AB907656	92%	*Kineococcus xinjiangensis* strain S2-20	NR_044522
22	70	Alphaproteobacteria	NyosB22 genotype 16	AB907657	100%	*Methylocystis echinoides* strain IMET 10491	NR_025544
23	80	Betaproteobacteria	NyosB23 genotype 17	AB907658	98%	*Thiobacillus thiophilus* strain D24TN	NR_044555
24	100	Firmicutes	NyosB24 genotype 18	AB907659	86%	*Desulfotomaculum australicum* strain AB33	NR_037008
25	100	Bacteroidetes	NyosB25 genotype 19	AB907660	88%	*Bizionia myxarmorum strain* ADA-4	NR_043121
26	120	Firmicutes	NyosB26 genotype 20	AB907661	85%	*Exiguobacterium antarcticum* B7	NR_074965
27	120	Ignavibacteriae	NyosB27 genotype 21	AB907662	90%	*Melioribacter roseus* P3M	NR_074796
28	120	Bacteroidetes	NyosB28 genotype 22_1	AB907663	93%	*Pedobacter terricola* strain DS-45	NR_044219
29	120	Actinobacteria	NyosB29 genotype 10_2	AB907664	93%	*Acidimicrobium ferrooxidans* DSM 10331	NR_074390
30	140	Firmicutes	NyosB30 genotype 23	AB907665	92%	*Clostridium cellulolyticum* H10	NR_102768
31	140	Firmicutes	NyosB31 genotype 24	AB907666	84%	*Bacillus alkalinitrilicus* strain ANL-iso4	NR_044204
32	160	Deltaproteobacteria	NyosB32 genotype 25	AB907667	91%	*Desulfovibrio vulgaris* DP4	NR_074897
33	160	Actinobacteria	NyosB33 genotype 10_3	AB907668	88%	*Acidimicrobium ferrooxidans* DSM 10331	NR_074390
34	160	Deltaproteobacteria	NyosB34 genotype 26_1	AB907669	92%	*Desulfovibrio alaskensis* G20	NR_074749
35	160	Bacteroidetes	NyosB35 genotype 22_2	AB907670	94%	*Pedobacter terricola* strain DS-45	NR_044219
36	180	Caldiserica	NyosB36 genotype 27_1	AB907671	90%	*Caldisericum exile* AZM16c01	NR_075015
37	180	Caldiserica	NyosB37 genotype 27_2	AB907672	93%	*Caldisericum exile* AZM16c01	NR_075015
38	180	Deltaproteobacteria	NyosB38 genotype 26_2	AB907673	91%	*Desulfovibrio alaskensis* G20	NR_074749
39	180	Nitrospirae	NyosB39 genotype 28	AB907674	93%	Candidatus *Nitrospira defluvii*	NR_074700
40	180	Bacteroidetes	NyosB40 genotype 22_3	AB907675	96%	*Pedobacter terricola* strain DS-45	NR_044219
41	180	Actinobacteria	NyosB41 genotype 10_4	AB907676	94%	*Acidimicrobium ferrooxidans* DSM 10331	NR_074390
42	180	Deltaproteobacteria	NyosB42 genotype 29	AB907677	87%	*Pelobacter acidigallici* strain DSM 2377	NR_026154
43	200	Actinobacteria	NyosB43 genotype 30	AB907678	91%	*Actinobaculum urinale* strain R9242	NR_028978
44	210	Firmicutes	NyosB44 genotype 31	AB907679	88%	*Catenibacterium mitsuokai* DSM 15897	NR_027526
45	210	Terenicutes	NyosB45 genotype 32	AB907680	90%	*Mycoplasma glycophilum* Strain 486	NR_025184
46	210 (Sed)	Fusobacteria	NyosB46 genotype 33	AB907681	99%	*Psychrilyobacter atlanticus* strain HAW-EB21	NR_042997

[a]NyosB1 to NyosB46 are the 46 sequenced bacterial bands and [b]Genotypes 1 to 33 indicate the 33 related genotypes.

Table 2 | Archaeal sequences identified at different depths of Lake Nyos and their closest matches to the 16S rRNA gene sequences for archaea database in the GenBank

		Band				Closest match	
No	Depth (m)	Phylum	Genotype[a,b]	Accession no.	Similarity	Organism	Accession no.
1	10	Thaumarchaeota	NyosA1 genotype 1_1	AB907765	89%	Candidatus *Nitrosopumilus koreensis* AR1	NR_102904
2	25	Thaumarchaeota	NyosA2 genotype 1_2	AB907766	89%	Candidatus *Nitrosopumilus koreensis* AR1	NR_102904
3	25	Thaumarchaeota	NyosA3 genotype 1_3	AB907767	89%	Candidatus *Nitrosopumilus koreensis* AR1	NR_102904
4	45	Thaumarchaeota	NyosA4 genotype 1_4	AB907768	91%	Candidatus *Nitrosopumilus koreensis* AR1	NR_102904
5	45	Thaumarchaeota	NyosA5 genotype 1_5	AB907769	94%	Candidatus *Nitrosopumilus koreensis* AR1	NR_102904
6	45	Thaumarchaeota	NyosA6 genotype 1_6	AB907770	89%	Candidatus *Nitrosopumilus koreensis* AR1	NR_102904
7	60	Thaumarchaeota	NyosA7 genotype 1_7	AB907771	90%	Candidatus *Nitrosopumilus koreensis* AR1	NR_102904
8	70	Thaumarchaeota	NyosA8 genotype 1_8	AB907772	90%	Candidatus *Nitrosopumilus koreensis* AR1	NR_102904
9	80	Euryarchaeota	NyosA9 genotype 2	AB907773	87%	*Methanolobus psychrophilus* R15	NR_102921
10	120	Euryarchaeota	NyosA11 genotype 3_1	AB917141	88%	*Natronolimnobius baerhuensis* IHC-005	NR_028161
11	120	Euryarchaeota	NyosA12 genotype 3_2	AB917142	92%	*Natronolimnobius baerhuensis* IHC-005	NR_028161
12	180	Euryarchaeota	NyosA10 genotype 3	AB907774	92%	*Natronolimnobius baerhuensis* IHC-005	NR_028161
13	210	Crenarchaeota	NyosA13 genotype 4_1	AB917143	80%	*Vulcanisaeta distributa* DSM 14429	NR_102943
14	210 (Sed)	Crenarchaeota	NyosA14 genotype 4_2	AB917144	80%	*Vulcanisaeta distributa* DSM 14429	NR_102943
15	210 (Sed)	Euryarchaeota	NyosA15 genotype 5	AB917145	91%	*Salinarchaeum* sp. Harcht-Bsk1	NR_103951

[a]NyosA1 to NyosA15 are the 15 sequenced archaeal bands and [b]Genotypes 1 to 15 indicate the 5 related genotypes.

producer detected in this study could be NyosB46 genotype 33 from the sediment sample since its sequence matched with *Psychrilyobacter atlanticus* strain HAW-EB21, a hydrogen (H_2) producer[27].

Archaeal sequences dominating from −10 to −70 m were closely related to the ammonia oxidising archaeon Candidatus *Nitrosopumilus koreensis* strain AR1. They inhabit various environments among which marine water, fresh water and hot springs and play important roles in the global nitrogen and carbon cycles[28–30]. The sequenced band from −80 m revealed a similarity of 87% to *Methanolobus psychrophilus* R15, a methanogen[31]. The sequences of bands from −120 and −180 m matched with *Natronolimnobius baerhuensis* IHC-005, a species isolated from soda lakes capable to reduce nitrate to nitrite and thiosulfate or sulfur to sulfide[32]. Bands from the −210 m (water and sediment sample) resulted in sequences matching with the Euryarchaeota *Vulcanisaeta distributa* DSM 14429 strain IC-017 and *Salinarchaeum* sp. Harcht-Bsk1.

Discussion

Bacterial and archaeal communities detected in this study showed a stratified distribution in the water column agreeing with previous studies using physico-chemical approaches. Several genera belonging to diverse functional groups were detected. This diversity of microbial communities likely reflects the uniqueness of Lake Nyos. However, it is worth mentioning that artifacts may be introduced in the microbial assemblages during DNA extraction, PCR or DGGE and therefore alter the natural diversity of microbial communities[33]. Consequently, the microbial community composition as detected by DGGE in this study may mainly represent the most abundant phylotypes. If functionally active, they could considerably impact the sulfur, hydrogen, nitrogen, methane, and carbon cycles taking place in the lake.

Altogether, the dendrograms showed clusters of the water stratification following the physico-chemical pattern indicated by Kusakabe *et al.*[8]; this clustering is also well illustrated by the MDS analysis with nearly similar clusters. Microorganisms require different metabolic pathways to survive and differences in concentrations of the chemical species may, to a large extent, explain differences in the bacterial and archaeal communities between the water layers. This remark is supported by the disposition of the sampling depths on relatively well defined loci on the PCA plot. The shallowest and deepest sampling sites are well separated, indicating the structuring role of the physicochemical parameters that have likely influenced the microbial community distribution.

The taxonomic structure of both bacterial and archaeal communities assemblage was nearly similar, with clear phylogenetic distribution influenced by the oxic or anoxic state of the sampling depths. Sequences found from 0 to −45 m were exclusively closer to aerobic species; from −55 to −180 m, they matched with both aerobic and anaerobic species and under −180 m, they were all close to anaerobes. The deep layers of Lake Nyos are known to be anoxic[8], as demonstrated by our measurements. The presence of such potential aerobic species might suggest that minute amounts of oxygen may be found up to deep water layers in the lake.

Apart from the CO_2 concentration, many other environmental variables were linked to the vertical stratification of the studied communities as typically seen in meromictic lakes. Sequences close to the phylogenetic groups Actinobacteria, Firmicutes, Nitrospirae and Proteobacteria were detected as in Lake Tanganyika[34]; archaeal sequences related to Thaumarchaeota were found in the upper part of the lake, as in the case of other meromictic lakes such as Lake Kivu[35,36] and Lake Ace[37] and marine environments such as the northern South China Sea[38].

Genetic fingerprinting techniques are key tools for studies of microbial communities, but do not provide direct evidence of a community's functional capabilities. Assurances on functionality of genes encoded in a microorganism's genome could only be reached through culture techniques or studies based on RNA analyses. However, functionality of the genome of a detected organism may be predicted on the basis of functions encoded in closely related genomes[39]. The microbial sequences retrieved from Lake Nyos water column were related to a variety of different functional groups of which a speculative correlation with the lake water's chemistry is discussed in the following lines.

Chemical reactions involving iron species in the lake could be governed by the combined contribution of sequences related to *A. ferrooxidans* strain DSM 10331 (at −45, −120, −160 and −180 m) and *F. thermotolerans* strain Y005 (at −55 m). The gradual increase of Fe^{2+} concentration towards the bottom of the lake as observed on Figure 2c could result from the concomitant actions of these iron related species. The vertical plot of Fe^{2+} concentration along the water column illustrates that iron is one of the most important chemical species in Lake Nyos. In fact, the iron cycle principally features the interchange of Fe^{2+} to Fe^{3+} and vice versa. With respect to the putative iron metabolising bacteria that were detected in the water column, both reactions would be expected to take place. But the increasing concentration of Fe^{2+} with depth suggests that the

most favorable reaction would be the reduction of Fe^{3+} to Fe^{2+}, which may contribute to the siderite formation at the bottom of the lake observed by Bernard and Symonds[10]. This hypothesis is supported by the study of Ellwood *et al.*[40] stating that the formation of siderite from Fe^{2+} produced during bacterial dissimilatory iron reduction is plausible in anoxic sediments. In fact, dissimilatory reduction occurs when ferric iron serves as a terminal electron acceptor during anaerobic respiration[41]. Under such conditions, pyrite mentioned by Bernard and Symonds[10] may likely form as well by using the sulfur species produced in the sulfur cycle.

Apart from the ability of these two phylogenetic groups to transform iron species, they have been reported to fix CO_2[42] as also may do the sequences related to *T. thiophilus* strain D24TN[15,18] at 80 m. If active in the Lake Nyos water column, such CO_2 fixing bacteria would likely contribute to the decrease of the CO_2 concentrations, even though at a micro level.

In the anoxic layers of Lake Nyos, several genotypes with putative activity in the sulfur cycle were detected. Such microorganisms have been reported in Lake Cadagno[43] and Lake Ace[37]. Sulfate ion is a primary factor in the distribution of microbial activities in anoxic sediments. When sulfate diffuses into anoxic habitats, it provides an opportunity for different groups of microorganisms to carry out dissimilatory sulfate reduction. This results in sulfide accumulation. Sulfide can serve as an electron source for anoxygenic chemolithoautotrophs such as *Thiobacillus*. SO_4^{2-} is a common source of energy for anaerobic sulfobacteria. These biological processes produce reduced sulfur species as well as elemental sulfur[44,45]. In our study, the genotypes that could putatively act on sulfur species are the archaeal sequences detected at −120 and −180m and the bacterial sequences detected at −80, −100, −160 m as previously mentioned. However, sulfur is depleted along the water column and this could limit the dissimilatory capacity of the sulfate related microorganisms[36,46]. If functional in the lake, they may degrade a wide variety of organic compounds heterotrophically or grow autotrophically, fixing inorganic CO_2 into central metabolic intermediates, given that they are metabolically versatile[47].

Two sequences close to species related to the methane metabolism were found at −70 and −80 m. The presence of methane in the lake have been mentioned and thought to be biogenic[12,48,49]. The NyosA9 genotype 2 could be a putative producer of the methane present in the lake, although its origin has not yet been determined. A non-sequenced band appears at the same position on the DGGE gel (archaea) for the sample collected at −90 m. We also specify that all the archaeal bands sequenced from −120 to −210 m matched with the unclassified anaerobic methanogenic archaeon ET1-10 in the nucleotide collection database. The similarity was quite high (86–99%) with respect to the similarity obtained in the 16S rRNA gene database for bacteria and archaea. This suggests that the organisms could correspond to a methanogenic archaeon, rather than *Vulcanisaeta distributa*. The detection of such putative methanogenic organism appears to answer the question of methane source in Lake Nyos. Safety concerns have been raised by Issa *et al.*[49] regarding the increasing concentration of CH_4 in the lake, since it has a low solubility. If the methanotrophic related bacteria detected are functionally active, their presence in the lake could be reassuring because they would feed on the available methane, contributing hereby to its reduction. Consequently, the methane present in the lake would be affected by methanogenesis and methanotrophy reactions performed by the detected microorganisms, subject to their functionality and abundance. The microbial methane production could also be influenced by the sulfur cycle. For instance, methanogenesis is responsible of the majority of terminal metabolism under anoxic conditions in freshwaters. However, methanogenesis and sulfate respiration compete for the same substrates. In such conditions, sulfate respiration likely dominates on the methanogenesis[50,51]. When enough sulfate-reducing bacteria are present, they maintain the concentrations of hydrogen and acetate at levels too low for methanogens to grow[52]. Part of the methane could also be oxidised into HCO_3^- and HS by the putative sulfate-reducing bacteria[53] detected in the lake. Taking into account these hypotheses and the presence of methanotrophic bacteria, we could expect that the methane concentration is kept low. A specific study using primers to screen methane metabolising archaea and bacteria, as well as quantitative analyses of methane and sulfur bacteria, would be needed to test these hypotheses.

Na^+ concentrations increased towards the bottom of the lake, with the highest concentrations in the deepest layers (−207 to −210 m); the sequence of one strong band of the bottom sample matched with *Salinarchaeum* sp. strain HArcht-Bsk1 (91%). Together with the sequences from −120 and −180 m, matching with *N. baerhuensis* IHC-005 (88–92%), they constitute putative halobacteriaceae species that could exist in the deepest layers of Lake Nyos. *Salinarchaeum* sp. strain HArcht-Bsk1 was isolated from Lake Baskunchak (Russia), with a hypersaline chloride-sulfate environment[54]; and *N. baerhuensis* IHC-005 from soda lakes in Inner Mongolia[32].

Several other functions such as the phosphate metabolism could also take place in the lake water column as related organisms (NyosB7 genotype 3_1 and NyosB12 genotype 3_2) close to *M. glycogenica*[13] were detected.

We used molecular techniques to study the microbial diversity in a meromictic lake water column and sediments. The archaeal and bacterial communities were revealed for the first time and showed a stratified pattern that mapped with the limnological conditions of the lake. The potential interactions of these communities with the chemical species of the lake's water column have been discussed. This study is a starting point of a broad descriptive microbiology in Cameroon's lakes and other water ecosystems. Many putative biotechnologically interesting microorganisms, with genes sufficiently different (from their closest matches in the GenBank) to be novel species candidates have been detected. This pioneer inventory will serve as a guide for further studies. The sequences corresponding to potential CO_2, CH_4, or Fe metabolising bacteria/archaea could be used in the bio-monitoring of these chemically important species of the lake. Biotechnological use in the removal of CO_2 could be one of the future interests.

However, this study may have been affected by technical limitations, such as biases that could be introduced by PCR or DGGE. Therefore, we obviously have got the bacterial community as determined by DGGE, but this does not insure the disclosure of the whole bacterial and archaeal communities. High throughput studies using sharper methods including next generation sequencing would provide more complete information on the microbial communities; furthermore, a three-pronged analytical approach based on the 16S rRNA studies, genes quantification and specific detection is recommended to clarify the functionality and abundance of the species and to characterise the organisms responsible of key biogeochemical functions taking place in the lake.

Methods

Site description. Lake Nyos is a crater lake located in the Northwest of Cameroon. It lies within the Oku volcanic field, along the Cameroon Volcanic Line (CVL) which runs from the Atlantic Ocean to the interior of Cameroon (Figure 1). The volcanism of the CVL is mostly basaltic and dated about 4,000 years[55].

Sample collection and processing. The samples were collected at the end of the dry season (March 2013) from the centre of the lake (N06°26′23.0″ and E10°18′02.3″) every 10, 15 or 20 m depth along the water column using a 1.6 l Niskin bottle. Each sample was transferred to a 1 l sterile polypropylene bottle and immediately kept in an ice-cooled box. Subsequently, they were vacuum filtered with a 0.22 μm membrane filter, then stored frozen until DNA extraction. Simultaneously with the water sampling, a profile of the water column was done using a conductivity-temperature-depth (CTD, Ocean Seven 316, Idronaut, Italy) profiler fitted with sensors to measure the conductivity, temperature, pressure (which is later converted to depth), pH and redox potential. DO concentration was measured at the depths of 100, 120, 140, 180, 200 and 210 m using the modified Winkler titration method[56].

DNA extraction. Total DNA was extracted from the membrane filters using a soil sample DNA extraction kit (UltraClean® Soil DNA Isolation Kit; Mo Bio Laboratories, CA, USA) and following the manufacturer's instructions. The genomic DNA was then quantified with a spectrophotometer (Nanodrop 1000 Spectrophotometer; Thermo Fisher Scientific K.K., Kanagawa, Japan) and stored at −20°C for later use.

Small Subunit rRNA gene amplification, DGGE analysis and sequencing. PCR amplification was performed for the domains bacteria and archaea in a 40 µl volume containing approximately 5 ng template DNA, 200 mmol l^{-1} of each of the deoxynucleoside triphosphates, 250 µmol l^{-1} of each of the primers, 1x Ex-Taq buffer and 0.5 U of DNA Taq polymerase (Takara Bio, Inc., Shiga, Japan). More information on the primers used in this study is given in supplementary Table S2. To analyse the bacterial diversity, the universal primers GC-341f and 518r[57] were used under the following thermal cycles: the initial denaturation at 94°C for 3 min; 20 touchdown cycles of denaturation (at 98°C for 10 sec), annealing (at 65°C for 30 sec, decreasing 0.5°C each cycle) and extension (at 72°C for 30 sec); 15 standard cycles of denaturation (at 98°C for 10 sec), annealing (at 55°C for 30 sec, and extension (at 72°C for 30 sec), and a final extension at 72°C for 3 min. For the archaeal 16S rRNA genes, a nested PCR using the primers sets 20f and 958r[57] in the first round of PCR and the inner primers GC-340f and 519r[57] in the second round with the following for both: the initial denaturation at 94°C for 3 min; 10 touchdown cycles of denaturation (at 94°C for 1 min), annealing (at 53°C for 1 min, decreasing 1°C each cycle) and extension (at 72°C for 3 min). Successful DNA amplification was verified by electrophoresis of 2 µl of PCR products in 2% agarose gel in TAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.0) with a DNA Mass Ladder Standard (Nippon Gene, Tokyo, Japan).

The remaining 38 µl of the amplified 16S rRNA gene fragments were analysed by DGGE with a Dcode™ Population Base System (Bio-Rad, Laboratories, CA, USA). PCR products were separated with an 8% acrylamide gel on a linear gradient of the denaturants urea and formamide increasing from 25% at the top of the gel to 60% at the bottom and ran as described by Muyzer *et al.*[58] with little modifications. Samples were analysed simultaneously with a marker at both extreme wells. The gel was stained with ethidium bromide and documented using a UV-transilluminator (MultiDoc-It™ Digital Imaging System; UVP, CA, USA) and a C-5060 Wide Zoom imaging systems (Olympus, Tokyo, Japan).

DGGE pattern analysis, phylogenetic analysis of excised bands and statistical analysis. The positions and relative signal intensities of detected bands in each gel track were determined with FP Quest software (Bio-Rad, Laboratories, CA, USA). Relative signal intensities were calculated from the peak area of the densitometric curves. Clustering analysis of the DGGE banding pattern was performed with the unweighted pair-group method using arithmetic averages (UPGMA), and the FP Quest software package was used for dendrogram construction. The Shannon-Weaver index (*H'*) was used as an estimate of microbial diversity. *H'* was calculated using the following equation[59]:

$$H' = \sum_{i=0}^{R} Pi \ \ln \ Pi$$

Where, *pi* represents the relative signal intensities of bands in a track and *R*, the Richness. All the detected bands were used for the calculation of diversity indices.

We applied principal component analysis (PCA) and the MDS algorithm to explore variation in the data with the PRIMER software (version 2, PRIMER-E Ltd, Plymouth, UK). For the PCA, the data matrix used the physico-chemical parameters as the variables at each depth. For the MDS, depths were used as the variables, the band scores as the values within each variable, and the correlation coefficient to calculate the similarity matrix. The first two components (Supplementary Table S3) were used to interpret the results.

The strong bands were stabbed with a sterile pipette tip; each stab was placed into 1.5 ml eppendorf tubes with 100 µl of sterile water and stored overnight at 4°C. After an amplification check, the bands were purified on Qiaquick columns (Qiagen, Tokyo, Japan) and a BigDye PCR (Applied Biosystems, CA, USA) performed. Then they were sequenced on an ABI-Prism sequencer (Applied Biosystems) using the reverse primer 518r for bacterial sequences and 519r for archaeal sequences. The obtained sequences were manually edited in the ATGC software. Subsequently, they were submitted to the GenBank database of the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov) for alignment employing the Basic Local Alignment Search Tool (BLASTN) algorithm of nucleotide in the 16S rRNA sequences (Bacteria and Archaea) database to determine their phylogenetic affiliations. The nearest neighbours of the submitted sequences, with the lowest E-value were considered as closest taxonomic affiliates.

Nucleotide sequence accession numbers. The sequences generated in this study were deposited at the GenBank/EMBL/DDBJ databases under the accession numbers AB907636 to AB907681 for bacteria; AB907765 to AB907774, and AB917141 to AB917145 for archaea.

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Author contributions

This work was conceived by R.E.T., S.N. and A.U. The sampling was done by R.E.T., W.F., M.K., G.T., B.T. and V.H. M.K. and T.O. generated the physico-chemical data. Molecular analyses were done by R.E.T., A.S., A.N. and D.T. The manuscript was written and approved by all the authors.

Additional information

Supplementary information accompanies this paper at http://www.nature.com/scientificreports

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