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; 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. 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”. They are at the base of the foodwebs in numerous environments where their several metabolisms mobilise the energy. 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. 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. 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. Its water column is divided into layers separated by vertical gradients of temperature and dissolved chemical species. The CO₂ from deep-magmatic origin reaches the bottom and dissolves into the lake water. 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 CO₂ 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. The dissolved chemical species are overwhelmingly dominated by CO₂ and HCO₃⁻, followed by Fe²⁺. 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. In the aftermath of the gas explosion at Lake Nyos, the works of Kling et al. and Kusakabe et al. 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.
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 245 to 280 m (Figure 2b). The Fe$^{2+}$ concentration was low from 0 to 280 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
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 occurring 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 255 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 Thaumarcheota (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 CO₂, HCO₃⁻, Na⁺ and Fe²⁺ while smaller changes were observed for Mn²⁺, SO₄²⁻, NO₃⁻ and SiO₂ concentrations. NO₃⁻ 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 physicochemical 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.
and capable to reduce nitrate as mentioned by Shintani et al.\textsuperscript{13} were also present. Organisms close to the Acidimicrobium ferrooxidans, capable of ferrous iron oxidation and carbon dioxide fixation\textsuperscript{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\textsuperscript{15}. Melioribacter roseus strain P3M may also be able to use ferric iron as electron acceptor as mentioned by Kadnikov et al.\textsuperscript{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 $-70$ m; the latter is an anaerobic organism capable of methane-oxidation\textsuperscript{17}. Sequences matching with the thiosulfate oxidising and carbon dioxide fixing bacteria Thiobacillus thiophilus strain D24TN\textsuperscript{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 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.

**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.
in the thiosulfate metabolism as they matched with *Caldisericum exile* strain AZM16c01T isolated from a hot spring in Japan and reported as thiosulfate-reducing bacterium\(^1\). 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.\(^2\), Love et al.\(^3\) and Hauser et al.\(^4\), 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.\(^5\), Sorokin et al.\(^6\) and Podosokorskaya et al.\(^7\), respectively were detected. One of the bands sequenced at −140 m was the close relative of *Clostridium cellulolyticum* H10, reported to produce H\(_2\).\(^8\) Another putative hydrogen
| No | Depth (m) | Phylum          | Genotype  | Accession no. | Similarity | Closest match                                                                 | Accession no. |
|----|-----------|-----------------|-----------|---------------|------------|---------------------------------------------------------------------------------|--------------|
|    |           |                 | Band      |               |            | Exiguobacterium undae strain DSM 14481                                           | NR_043477    |
| 1  | 0         | Firmicutes      | NyosB1 genotype 1_1 | AB907636 | 100%       |                                                                                  |              |
| 2  | 0         | Firmicutes      | NyosB2 genotype 1_2 | AB907637 | 100%       |                                                                                  |              |
| 3  | 0         | Firmicutes      | NyosB3 genotype 1_3 | AB907638 | 100%       |                                                                                  |              |
| 4  | 0         | Actinobacteria  | NyosB4 genotype 2 | AB907639 | 97%        | Solirubrobacter soli strain Gsoil 355                                           | NR_041365    |
| 5  | 0         | Firmicutes      | NyosB5 genotype 1_4 | AB907640 | 100%       |                                                                                  |              |
| 6  | 0         | Firmicutes      | NyosB6 genotype 1_5 | AB907641 | 99%        |                                                                                  |              |
| 7  | 0         | Actinobacteria  | NyosB7 genotype 3_1 | AB907642 | 93%        |                                                                                  |              |
| 8  | 0         | Firmicutes      | NyosB8 genotype 4_1 | AB907643 | 99%        |                                                                                  |              |
| 9  | 0         | Firmicutes      | NyosB9 genotype 4_2 | AB907644 | 97%        |                                                                                  |              |
| 10 | 0         | Firmicutes      | NyosB10 genotype 5 | AB907645 | 100%       |                                                                                  |              |
| 11 | 0         | Firmicutes      | NyosB11 genotype 6 | AB907646 | 95%        |                                                                                  |              |
| 12 | 0         | Actinobacteria  | NyosB12 genotype 3_2 | AB907647 | 93%        |                                                                                  |              |
| 13 | 35        | Gammaproteobacteria | NyosB13 genotype 7 | AB907648 | 100%       |                                                                                  |              |
| 14 | 35        | Actinobacteria  | NyosB14 genotype 8 | AB907649 | 100%       |                                                                                  |              |
| 15 | 45        | Actinobacteria  | NyosB15 genotype 9 | AB907650 | 93%        |                                                                                  |              |
| 16 | 45        | Actinobacteria  | NyosB16 genotype 10_1 | AB907651 | 92%        |                                                                                  |              |
| 17 | 55        | Actinobacteria  | NyosB17 genotype 11 | AB907652 | 94%        |                                                                                  |              |
| 18 | 60        | Gammaproteobacteria | NyosB18 genotype 12 | AB907653 | 99%        |                                                                                  |              |
| 19 | 60        | Betaproteobacteria | NyosB19 genotype 13 | AB907654 | 99%        |                                                                                  |              |
| 20 | 60        | Betaproteobacteria | NyosB20 genotype 14 | AB907655 | 95%        |                                                                                  |              |
| 21 | 70        | Actinobacteria  | NyosB21 genotype 15 | AB907656 | 92%        | Kineococcus xinjiangensis strain S2-20                                           | NR_044522    |
| 22 | 70        | Alphaproteobacteria | NyosB22 genotype 16 | AB907657 | 100%       |                                                                                  |              |
| 23 | 80        | Betaproteobacteria | NyosB23 genotype 17 | AB907658 | 98%        | Thiobacillus thiophilus strain D24TN                                             | NR_044555    |
| 24 | 100       | Firmicutes      | NyosB24 genotype 18 | AB907659 | 86%        | Desulfotomaculum auripullorum strain AB33                                        | NR_037008    |
| 25 | 100       | Bacteroidetes   | NyosB25 genotype 19 | AB907660 | 88%        | Bizonia myxamororum strain ADA4                                                 | NR_043121    |
| 26 | 120       | Firmicutes      | NyosB26 genotype 20 | AB907661 | 88%        | Exiguobacterium undae strain DSM 14481                                           | NR_043477    |
| 27 | 120       | Ignavibacteriae | NyosB27 genotype 21 | AB907662 | 90%        | Melobacter roseus P3M                                                            | NR_074976    |
| 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 strain H10                                            | NR_102768    |
| 31 | 140       | Firmicutes      | NyosB31 genotype 24 | AB907666 | 84%        | Bacillus alkaliarchaeis strain ANL-60                                           | NR_044204    |
| 32 | 160       | Deltaproteobacteria | NyosB32 genotype 25 | AB907667 | 91%        | Desulfuvirbio vulgaris strain DP4                                                 | NR_074897    |
| 33 | 160       | Actinobacteria  | NyosB33 genotype 10_3 | AB907668 | 88%        | Desulfosulfobacterium ferrooxidans DSM 10331                                     | NR_074390    |
| 34 | 160       | Deltaproteobacteria | NyosB34 genotype 26_1 | AB907669 | 92%        | Desulfuromonas alaskensis strain G20                                              | NR_074749    |
| 35 | 160       | Bacteroidetes   | NyosB35 genotype 22_2 | AB907670 | 94%        | Pedobacter terricola strain DS-45                                               | NR_044219    |
| 36 | 180       | Caldiseriacaeae | NyosB36 genotype 27_1 | AB907671 | 90%        | Caldibergeria exii strain DSM 16577                                              | NR_075015    |
| 37 | 180       | Caldiseriacaeae | NyosB37 genotype 27_2 | AB907672 | 93%        | Caldibergeria exii strain DSM 16577                                              | NR_075015    |
| 38 | 180       | Deltaproteobacteria | NyosB38 genotype 26_2 | AB907673 | 91%        | Desulfuromonas alaskensis strain G20                                              | NR_074749    |
| 39 | 180       | Nitrospirae     | NyosB39 genotype 28 | AB907674 | 93%        | Candidatus Nitrospira defluviensis strain                                        | 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%        | Pedobacter acidilagillici strain DSM 2377                                        | NR_026154    |
| 43 | 200       | Actinobacteria  | NyosB43 genotype 30 | AB907678 | 91%        | Actinobacillus urinale strain R9242                                              | NR_028978    |
| 44 | 210       | Firmicutes      | NyosB44 genotype 31 | AB907679 | 88%        | Catenibacterium misuokai 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%        | Psychrophilax bacillus arcticus strain HAW-EB21                                  | NR_042997    |

*NyosB1 to NyosB46 are the 46 sequenced bacterial bands and *Genotypes 1 to 33 indicate the 33 related genotypes.
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, 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 CO₂ 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]

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### 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. The microbial assemblages during DNA extraction, PCR or DGGE likely reflects the uniqueness of Lake Nyos.

Several genera belong to the sediment sample since its sequence matched with *Psychroballus atlanticus*. The producer detected in this study could be NyosB46 genotype 33 from the sediment sample since its sequence matched with *Psychroballusatlanticus* strain HAW-EB21, a hydrogen (H₂) producer.[33]

Archaeal sequences dominating from −10 to −70 m were closely related to the ammonia oxidising archaeon *Candidatus Nitrospumilus 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.[34-36] The sequenced band from −80 m revealed a similarity of 87% to *Methanobacterium psychrophilus* R15, a methanogen.[41] The sequences of bands from −120 and −180 m matched with *Natronolimnobius baerhuensis* IFC-005, a species isolated from soda lakes capable to reduce nitrate to nitrite and thiosulfate or sulfur to sulfide.[42] 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.

### 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

| No | Depth [m] | Phylum | Genotypeᵃᵇ | Accession no. | Similarity | Organism Accession no. |
|----|----------|--------|------------|--------------|-----------|------------------------|
| 1  | 10       | Thaumarchaeota | NyosA1 genotype 1_1 | AB907765 | 89% | *Candidatus Nitrospumilus koreensis* AR1 NR_102904 |
| 2  | 25       | Thaumarchaeota | NyosA2 genotype 1_2 | AB907766 | 89% | *Candidatus Nitrospumilus koreensis* AR1 NR_102904 |
| 3  | 25       | Thaumarchaeota | NyosA3 genotype 1_3 | AB907767 | 89% | *Candidatus Nitrospumilus koreensis* AR1 NR_102904 |
| 4  | 45       | Thaumarchaeota | NyosA4 genotype 1_4 | AB907768 | 91% | *Candidatus Nitrospumilus koreensis* AR1 NR_102904 |
| 5  | 45       | Thaumarchaeota | NyosA5 genotype 1_5 | AB907769 | 94% | *Candidatus Nitrospumilus koreensis* AR1 NR_102904 |
| 6  | 45       | Thaumarchaeota | NyosA6 genotype 1_6 | AB907770 | 89% | *Candidatus Nitrospumilus koreensis* AR1 NR_102904 |
| 7  | 60       | Thaumarchaeota | NyosA7 genotype 1_7 | AB907771 | 90% | *Candidatus Nitrospumilus koreensis* AR1 NR_102904 |
| 8  | 70       | Thaumarchaeota | NyosA8 genotype 1_8 | AB907772 | 90% | *Candidatus Nitrospumilus koreensis* AR1 NR_102904 |
| 9  | 80       | Euryarchaeota | NyosA9 genotype 2_1 | AB907773 | 87% | *Methanobacterium psychrophilus* R15 NR_102921 |
| 10 | 120      | Euryarchaeota | NyosA11 genotype 3_1 | AB917141 | 88% | *Natronolimnobius baerhuensis* IFC-005 NR_028161 |
| 11 | 120      | Euryarchaeota | NyosA12 genotype 3_2 | AB917142 | 92% | *Natronolimnobius baerhuensis* IFC-005 NR_028161 |
| 12 | 180      | Euryarchaeota | NyosA10 genotype 3_3 | AB907774 | 92% | *Natronolimnobius baerhuensis* IFC-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_5 | AB917145 | 91% | *Salinarchaeum* sp. Harcht-Bsk1 NR_103951 |

ᵃNyosA1 to NyosA15 are the 15 sequenced archaeal bands andᵇGenotypes 1 to 15 indicate the 5 related genotypes.

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 CO₂ 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]

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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. thermostolerans* strain Y005 (at −55 m). The gradual increase of Fe²⁺ 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 Fe²⁺ 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 Fe²⁺ to Fe³⁺ 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 Fe³⁺ with depth suggests that the
most favorable reaction would be the reduction of Fe$^{2+}$ 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.$^{14}$ 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$^{17}$. 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$_2$ as also may do the sequences related to T. thiophilus strain D24TN$^{44,45}$ at 80 m. If active in the Lake Nyos water column, such CO$_2$ fixing bacteria would likely contribute to the decrease of 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 common source of energy for anaerobic sulfobacteria. These biological processes produce reduced sulfur species as well as elemental sulfur$^{46,47}$. 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 sulfated microorganisms$^{48-50}$. 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$^{51}$.

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$^{52-54}$. 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 archaenal 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 archaean, rather than *Vulcanaisaeta distributa*. The detection of such putative methanogenic organisms appears to answer the question of methane source in Lake Nyos. Safety concerns have been raised by Issa et al.$^{55}$ 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$^{56,57}$. When enough sulfate-reducing bacteria are present, they maintain the concentrations of hydrogen and acetate at levels too low for methanogens to grow$^{58}$. Part of the methane could also be oxidised into HCO$_3^-$ and HS by the putative sulfate-reducing bacteria$^{59}$ 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. baeruensis* IHC-005 (88–92%), they constitute putative halobacteriaeae 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$^{60}$; and *N. baeruensis* 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$^{51}$ were detected.

We used molecular techniques to study the microbial diversity in a meromictic lake water column and sediments. The archaean 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 bio-technologically 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$_4$, 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 archaean 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 lives 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 volcano of the CVL is mostly basaltic and dated about 4,000 years$^{62}$.

**Sample collection and processing.** The samples were collected at the end of the dry season (October 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 516, 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$^{63}$.
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 deoxyinosine triphosphates, 250 μmol l⁻¹ of each of the primers, 1X Ex-Taq buffer and 0.5 μl 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 amplify the bacterial 16S rRNA gene, a nested PCR using the primers sets 20f and 958rr in the first round of PCR and the inner primers GC-340F and 1519rr in the second round with the following for both: 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, successful 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).

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-Wiener index (H') was used as an estimate of microbial diversity. H' was calculated using the following equation:

\[ H' = -\sum_{i=1}^{R} P_i \ln P_i \]

Where, \( P_i \) 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 used 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 stained with a sterile pipette tip; each stab was placed into 1.5 ml ependorf 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, USA) was performed. Then they were sequenced on an ABI-Prism sequencer (Applied Biosystems) and a C-5060 Wide Zoom imaging systems (Olympus, Tokyo, Japan).

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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 Y.H. M.K. and T.G. 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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