Chapter 6
Microbial Ecology of Lake Kivu

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Abstract We review available data on archaea, bacteria and small eukaryotes in an attempt to provide a general picture of microbial diversity, abundances and microbe-driven processes in Lake Kivu surface and intermediate waters (ca. 0–100 m). The various water layers present contrasting physical and chemical properties and harbour very different microbial communities supported by the vertical redox structure. For instance, we found a clear vertical segregation of archaeal and bacterial assemblages between the oxic and the anoxic zone of the surface waters. The presence of specific bacterial (e.g. Green Sulfur Bacteria) and archaeal (e.g. ammonia-oxidising archaea) communities and the prevailing physico-chemical conditions point towards the redoxcline as the most active and metabolically diverse water layer. The archaeal assemblage in the surface and intermediate water column layers was mainly composed by the phylum *Crenarchaeota*, by the recently defined phylum *Thaumarchaeota* and by the phylum *Euryarchaeota*. In turn, the bacterial assemblage comprised mainly ubiquitous members of planktonic assemblages of freshwater environments (*Actinobacteria*, *Bacteroidetes* and *Betaproteobacteria*

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among others) and other less commonly retrieved phyla (e.g. *Chlorobi*, *Clostridium* and *Deltaproteobacteria*). The community of small eukaryotes (<5 μm) mainly comprised *Stramenopiles*, *Alveolata*, *Cryptophyta*, *Chytridiomycota*, *Kinetoplastea* and *Choanoflagellida*, by decreasing order of richness. The total prokaryotic abundance ranged between 0.5×10^6 and 2.0×10^6 cells mL^{-1}, with maxima located in the 0–20 m layer, while phycoerythrin-rich *Synechococcus*-like picocyanobacteria populations were comprised between 0.5×10^5 and 2.0×10^5 cells mL^{-1} in the same surface layer. Brown-coloured species of Green Sulfur Bacteria permanently developed at 11m depth in Kabuno Bay and sporadically in the anoxic waters of the lower mixolimnion of the main basin. The mean bacterial production was estimated to 336 mg C m^{-2} day^{-1}. First estimates of the re-assimilation by bacterioplankton of dissolved organic matter excreted by phytoplankton showed high values of dissolved primary production (ca. 50% of total production). The bacterial carbon demand can totally be fuelled by phytoplankton production. Overall, recent studies have revealed a high microbial diversity in Lake Kivu, and point towards a central role of microbes in the biogeochemical and ecological functioning of the surface layers, comprising the mixolimnion and the upper chemocline.

6.1 Introduction

Microbes include all organisms smaller than about 100 μm, which can be seen and/or analysed with a microscope (Kirchman 2008). These organisms include viruses, bacteria, archaea, and single-celled eukaryotes (protists). They are present in almost every environment on Earth spanning from the upper layers of the atmosphere to several kilometres below Earth's surface carrying out different types of metabolisms and consequently being involved in nearly all biogeochemical cycles (Kirchman 2008). According to their ubiquity, activity and large numbers, microbes are central players in nutrient cycling (Lindeman 1942; Cotner and Biddanda 2002).

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However, very few studies to date have addressed the diversity and function of microbes in large tropical lakes (Descy and Sarmento 2008). This under-sampling has prevented progress on topics such as microbial biogeography and comparative studies of lake microbial assemblages along latitudinal gradients or between temperate and tropical surface waters. The situation is similar when addressing the roles of microbes in ecosystem functioning and productivity in tropical lakes. Therefore, recently conducted research on the microorganisms of Lake Kivu is highly valuable and will be of use for future cross-system comparisons. The purpose of this chapter is to summarize the existing data on microbial assemblages in Lake Kivu and to provide a framework to interpret the microbial diversity present in this lake. We also discuss the implications of microbes in the major biogeochemical processes operating in the water column, and give currently available numbers of abundance and production.

The ecology of microorganisms from nanoplankton (i.e., with a median size from 2 to 20 μm) and microplankton (20–200 μm) is detailed in Chap. 5. In this chapter, we focus only on picoplankton, i.e. the planktonic organisms with a mean size <2 μm, and on single-celled eukaryotes smaller than 5 μm.

6.2 Lake Kivu and Potential Microbial Processes in Upper and Intermediate Water Layers

The vertical structure of the water column of Lake Kivu is peculiar and complex (more details on general physical and chemical characteristics are given in Chaps. 2, 3 and 4). In short, the surface waters alternate between deep mixing, down to ~65 m, during the dry season and a comparatively long stratification period during the rainy season. This surface layer, the mixolimnion, is separated from the deep waters, the monimolimnion, by a permanent chemocline located at ~65 m. Waters from the monimolimnion are always anoxic and rich in carbon dioxide, methane (CH_4), salts and nutrients (ammonium, NH_4^+, and phosphates) (Chaps. 3 and 10). By contrast, the surface waters are oxygenated and have very low nutrient concentrations. This illustrates the oxidation-reduction (redox) biogeochemical structure within the vertical profile, which sustains various microbial activities and communities.

Observations of the dynamics of the reduced forms of carbon (C), nitrogen (N) and sulfur (S) may help to identify the main microbial processes taking place in these upper layers of Lake Kivu water column. During the dry, windy season, the upper 60–65 m of the water column is well ventilated and mixed. This layer, called the mixolimnion, then contains oxygen with concentrations near the saturation, whereas deeper waters are anoxic and contain reduced forms of N (NH_4^+), C (CH_4) and S (hydrogen sulfide ion, HS^-) (Degens et al. 1973; Pasche et al. 2009). Most chemolithotrophic microbes typically grow at redox interfaces where anoxic water containing reduced substances come into contact with O_2 via water flow or molecular diffusion (Burgin et al. 2011). In Lake Kivu, O_2-driven CH_4 oxidation by aerobic methanotrophs is the major process reducing CH_4 concentrations in surface waters

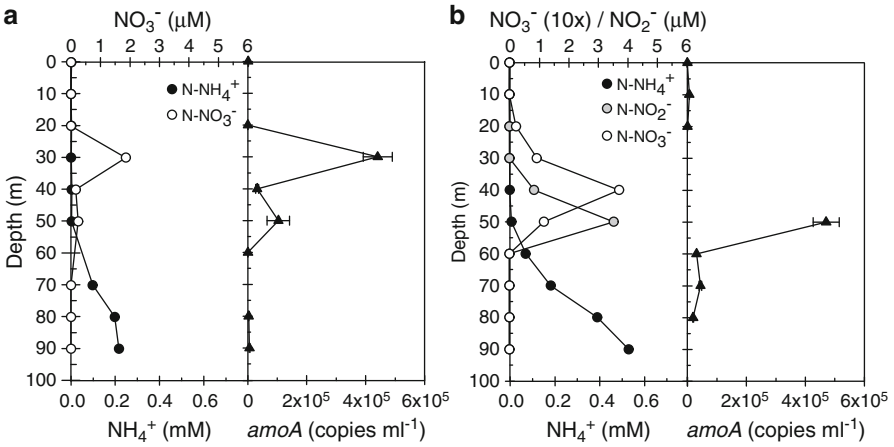

Fig. 6.1 Vertical depth profiles of nitrogen species (left panels) and archaeal ammonia monooxygenase subunit A (*amoA*) gene copy numbers (right panels) in the water column off Kibuye (**a**) and in the Ishungu basin (**b**) in March 2007 (Data extracted from Llirós et al. 2010)

(Pasche et al. 2011; Borges et al. 2011). Methanotrophy may also be performed anaerobically by archaea that oxidize CH_4 to obtain energy using sulfate (SO_4^{2-}) as the electron acceptor (Boetius et al. 2000; Orphan et al. 2001). In Lake Kivu, the inverse profiles of CH_4 and SO_4^{2-} in the intermediate zone from 60 to 90 m (Pasche et al. 2011) suggest the relevance of this process. Nevertheless, the sulfate flux budget indicates that only 3% of the CH_4 would be oxidized with SO_4^{2-} (Pasche et al. 2009, 2011). This estimate is a maximum because sulfate reducers can also use organic matter as an electron donor, pursuing anaerobically the heterotrophic decomposition of settling phytoplankton. The input fluxes of other potential electron acceptors for anaerobic CH_4 oxidation, i.e. nitrate, and oxidized iron and manganese ions, are too low in Lake Kivu to significantly oxidize CH_4 (Pasche et al. 2011).

Inputs of nutrients to surface waters by vertical mixing during the dry season favour phytoplankton growth (Chap. 5). The thermal stratification starts in October-November. An immediate decrease of oxygen concentrations is then observed in the lower mixolimnion (from ~25–30 m to ~65 m) due to the decay of settling organic matter combined to oxidation of reduced species (CH_4, HS^-, NH_4^+) diffusing from the anoxic layer. Therefore, during the major part of the rainy season, a gradient from oxic to anoxic conditions is present in the mixolimnion. The temperature in the lower depths of the mixolimnion is typically ~23°C throughout the year. The low solubility of oxygen coupled with intense microbial metabolic rates at these temperatures justify why these waters become very rapidly anoxic (Lewis 2010). During the second half of the rainy season (February to May), a nitrogenous zone characterized by the accumulation of nitrite (NO_2^-) and nitrate (NO_3^-) is often observed in the lower layer of the mixolimnion (Fig. 6.1). It results from nitrification of ammonium released by decaying organic matter (Wimba 2008; Llirós et al. 2010). Consequently nitrification may substantially contribute to oxygen depletion in the

lower mixolimnion. Besides, denitrification and/or anammox might take place under anoxic conditions, with NO_3^-, NO_2^- and/or nitrous oxide (N_2O) diffusing from the nitrogenous zone and, for anammox, NH_4^+ diffusing from the monimolimnion. It is worth noting that the nutrient profiles in Lake Kivu resemble those from Lake Tanganyika, where a tight coupling of NH_4^+ liberation during denitrification-based mineralization and further NH_4^+ oxidation by anammox bacteria was demonstrated (Schubert et al. 2006).

In the intermediate 60–90 m zone, the upward (diffusing and advective) fluxes of HS^- are greater than the downward fluxes of SO_4^{2-} (Pasche et al. 2009). The inverse profiles of HS^- and SO_4^{2-} observed in this zone are mainly explained by O_2-driven sulfide oxidation. When the nitrogenous zone is present, SO_4^{2-} production might also be performed by microbes that use NO_3^- as electron acceptors (Burgin and Hamilton 2008). This microbial process has recently been observed in the chemocline of a permanently stratified temperate fjord (Jensen et al. 2009), along the West-African continental shelf (Lavik et al. 2009) and in the oxygen-minimum zone of the eastern tropical Pacific ocean (Canfield et al. 2010). Finally, we can also predict high rates of aerobic sulfide oxidation during mixing conditions, when anoxic waters enriched with sulfide are mixed with oxygenated waters from the surface. These events must be accompanied by substantial O_2 consumption.

From previous observations, several dissimilatory transformations of oxidation-reduction substances appear in the surface and intermediate water layers of Lake Kivu, leading to an original microbial energy economy (Burgin et al. 2011), which still should be clarified. Major and putative microbially driven biogeochemical processes are summarized in Table 6.1, with references to studies that have so far explored the microbial actors, processes and/or rates. At least two processes should further be explored as they potentially directly couple several elemental cycles in Lake Kivu: anaerobic CH_4 oxidation and NO_3^--driven SO_4^{2-} production.

6.3 Archaeal and Bacterial Assemblages

As the anoxic deep waters of Lake Kivu contain huge amounts of CH_4 (Schmitz and Kufferath 1955; Degens et al. 1973), early microbiology studies focused on microbes involved in the CH_4 cycle, reporting evidence of the presence and activity of CH_4-oxidizing bacteria (Deuser et al. 1973; Jannasch 1975; Schoell et al. 1988). Very recently, Pasche et al. (2011) studied the phylogenetic diversity of the microbial assemblage involved in the CH_4 cycle in Lake Kivu. They used sequences of the particulate CH_4 monooxygenase (*pmoA*) and methyl coenzyme M reductase (*mcrA*) functional genes as molecular markers for, respectively, aerobic methanotrophic bacteria and methanogenic or anaerobic methanotrophic archaea. All *pmoA* sequences were most closely related to *Methylococcus*. In turn, *mcrA* gene sequences were absent from surface samples but the retrieved clones from deeper samples belonged to three main clusters, related to the *Methanomicrobiales* and the archaeal anaerobic methanotrophic ANME-1 clade (Hallam et al. 2003).

Table 6.1 Synthesis of microbially mediated biogeochemical processes known or potentially present in the first 0–100 m water column of Lake Kivu with references to studies when available

Processes	Source of energy	Final electron acceptor	Involved elements	References	Main research
Known processes					
Oxygenic photosynthesis (algae and cyanobacteria)	Light[a]	$NADP^+$	C	Sarmento et al. (2006, 2007, 2008, 2009), Chap. 5	Biodiversity, C incorporation rates
Anoxygenic photosynthesis (Green Sulfur Bacteria)	Light[b]	$NADP^+$	C	This study	Abundance
Aerobic degradation of organic matter	Organic matter	O_2	C, N, P, S, etc	Pasche et al. (2010)	Sedimentation and settling rates
Aerobic CH_4 oxidation	CH_4	O_2	C	Jannasch (1975), Pasche et al. (2011)	CH_4 oxidation measurements, *pmoA* phylogenetic tree
Anaerobic CH_4 oxidation	CH_4	SO_4^{2-}	C, S	Llirós et al. (2010), Pasche et al. (2011)	*mcrA* and 16S rRNA phylogenetic tree
Nitrification	NH_4^+	O_2	N	Llirós et al. (2010)	*amoA* and 16S rRNA phylogenetic tree
Potential processes					
Anaerobic degradation of organic matter	Organic matter	SO_4^{2-}	C, S	Pasche et al. (2011)	Mineralization rates
Denitrification	Organic matter	NO_3^-, NO_2^-, N_2O	N	None	
Dissimilatory nitrate reduction to ammonium (DNRA)	CH_2O, H^+	NO_3^-, NO_2^-	C, N	None	
Anammox	NH_4^+	NO_2^-	N	None	
Sulfur oxidation	H_2S, S^0	O_2	S	None	
NO_3^--driven SO_4^{2-} production	H_2S, S^0	NO_3^-	N, S	None	

$NADP^+$ Nicotinamide adenine dinucleotide phosphate

[a]Uses H_2O as electron donor

[b]Uses H_2S as electron donor

Table 6.2 Relative abundances of bacteria and archaea quantified by CARD-FISH[a] in February 2007 in Lake Kivu. Data are expressed in percentages of total cells enumerated after DAPI staining (data from Llirós et al. 2010)

Basin	Depth (m)	*Bacteria* (%)	*Archaea* (%)
Goma/Gisenyi			
	10	94.8 ± 0.4	0.5 ± 0.1
	30	59.4 ± 9.4	2.5 ± 1.4
	40	87.6 ± 8.5	4.3 ± 2.0
	60	58.1 ± 2.7	0.3 ± 0.1
	85	46.2 ± 3.8	3.3 ± 1.8
Bukavu Bay			
	10	62.5 ± 6.4	0.6 ± 0.1
	30	93.5 ± 5.1	1.1 ± 0.5
	40	79.4 ± 2.9	4.6 ± 1.0
	50	93.2 ± 2.4	2.7 ± 0.4
	85	83.1 ± 8.9	2.5 ± 0.5

[a]CARD-FISH counts using specific probes (EUBI-II-III for *bacteria* and ARC915 for *archaea*)

The sharp oxycline and the oligotrophic nature of the lake's mixolimnion offer an optimal niche for the development of autotrophic nitrifying archaeal populations (Martens-Habbena et al. 2009). Llirós et al. (2010) reported the recovery in Lake Kivu of a large set of sequences affiliated to *Marine Crenarchaeota Group 1.1a* and assigned to a unique OTU (operational taxonomic unit) related to *Nitrosopumilus maritimus*, indicating the presence of active archaeal nitrifiers in the lake water column. Furthermore, the detection of archaeal ammonia monooxygenase subunit A (*amoA*) genes at the depths where maxima of nitrate and nitrite were observed in two basins of the lake (Fig. 6.1) points to the involvement of planktonic archaea in nitrification processes in the oxycline (Llirós et al. 2010). Although confirmation by *in situ* activity measurements is still needed, available data suggest an active contribution of ammonia-oxidizing archaea (AOA) in the N cycle of Lake Kivu.

During the rainy season of 2007, CARD-FISH (Catalyzed Reporter Deposition Fluorescence *in situ* Hybridization) analyses revealed a marked dominance of bacteria over archaea throughout the first 100 m of the water column with values ranging from 46% to 95% of total DAPI stained cells for bacteria and from 0.3% to 4.6% for archaea (Llirós et al. 2010, Table 6.2). The small contribution of archaea to the planktonic microbial community is a common trait for freshwater environments (Casamayor and Borrego 2009). In this regard, values below 10% of total prokaryotes have usually been reported for different freshwater lakes (Pernthaler et al. 1998; Jürgens et al. 2000; Llirós et al. 2011), although higher archaeal abundances have been reported (>20% of total cells) in some oligotrophic (Urbach et al. 2007; Auguet and Casamayor 2008) or oligomesotrophic (Callieri et al. 2009) freshwater lakes.

In spite of their general modest contribution to the microbial assemblage of lakes, archaea are microbes of interest due to their recently documented widespread distribution and contribution to global energy cycles (Schleper et al. 2005). In Lake Kivu, the

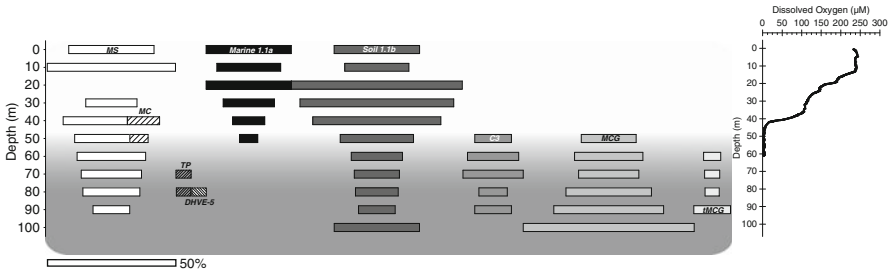

Fig. 6.2 Overview of the archaeal phylogenetic diversity of Lake Kivu water samples (extracted from Llirós et al. 2010). The width of the bar indicates, at each depth, the percentage of OTUs related to the indicated phylogenetic group. The oxygen gradient is plotted on the right. MS, *Methanosaeta*; MC, *Methanocella*; TP, *Thermoplasmata*; DHVE-5, Deep Hydrothermal Vent Euryarchaeota Group 5; Marine 1.1a, Marine Crenarchaeota Group 1.1a; Soil 1.1b, Soil Crenarchaeota Group 1.1b; C3, Crenarchaeota Group 1.2 or C3; MCG, Miscellaneous Crenarchaeotic Group or Crenarchaeota Group 1.3; tMCG, terrestrial Miscellaneous Crenarchaeotic Group

archaeal community in the surface and intermediate water column layers (ca. 0–100 m) during the rainy season 2007 was composed of phylotypes affiliated to the phylum *Crenarchaeota* (32% of the assigned OTUs), to the recently defined phylum *Thaumarchaeota* (43% of the assigned OTUs) and to the phylum *Euryarchaeota* (the remaining 25% of the assigned OTUs) (modified from Llirós et al. 2010). Within this latter phylum, most of the OTUs affiliated to methanogenic lineages, either acetoclastic (*Methanosaeta* spp.) or hydrogenotrophic ones (*Methanocellula* spp.), agreeing with the suggested biological origin of methane in the lake (Schoell et al. 1988; Pasche et al. 2011) and common methanogenic phylotypes present in stratified lakes (Lehours et al. 2007). Concerning *Thaumarchaeota* and *Crenarchaeota*, the phylotype richness showed a vertical structure of microbes related to the oxygen gradient. For the former, OTUs retrieved from the oxic water compartment mainly affiliated to *Marine Group 1.1a* (one single OTU with 95% sequence similarity to *N. maritimus*, a marine ammonia-oxidising archaea, Könneke et al. 2005) and to *Soil Group 1.1b* (some OTUs with high similarities to environmental sequences putatively involved in ammonia-oxidation, *e.g.* fosmid soil clone 54d9; Treusch et al. 2005), which are two lineages of the newly proposed phylum *Thaumarchaeota* containing all ammonia-oxidizing representatives known to date (Brochier-Armanet et al. 2008; Spang et al. 2010; Pester et al. 2011). All retrieved OTUs affiliated to the *Crenarchaeota* were retrieved from the anoxic water compartment with most of the sequences affiliated to lineages *Crenarchaeota Group 1.2* or C3 (DeLong and Pace 2001), the *Group 1.3* or *Miscellaneous Crenarchaeotic Group* (MCG, Inagaki et al. 2003) and the *terrestrial MCG* (tMCG, Takai et al. 2001), with yet unknown community role (Fig. 6.2).

In contrast with the studies of the archaeal assemblage, analyses on bacterial diversity are still scarce and the only available information comes from three recent studies (Libert 2010; Pasche et al. 2011; Schmitz 2011). Using denaturing gradient gel electrophoresis (DGGE) and 16S rRNA sequencing, Schmitz (2011) found a band distribution pattern coherent with the different water layers (Fig. 6.3, Table 6.3),

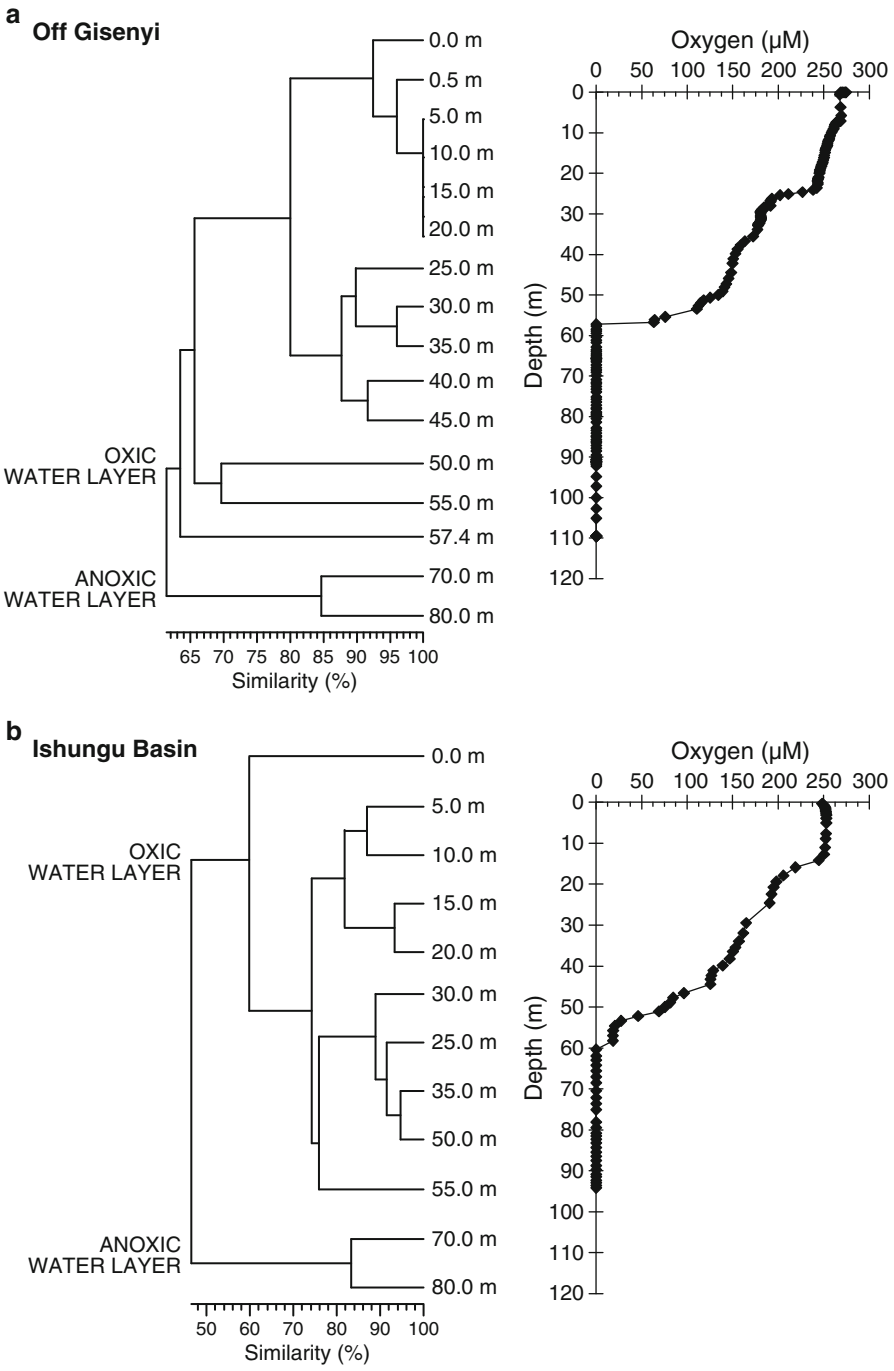

Fig. 6.3 Dendrograms based on Euclidean distances and unweighted pair-group method with arithmetic mean obtained from absence/presence matrices of bands extracted from bacterial 16S rRNA gene DGGE fingerprints from Lake Kivu water samples of October 2010 (rainy season) (from Schmitz 2011). Dissolved oxygen was measured during sampling

Table 6.3 Bacterial and archaeal operational taxonomic units (OTUs) retrieved after DGGE analysis of Lake Kivu water samples and general distribution across the different water layers (bacterial data from Schmitz 2011 and archaeal data from Llirós et al. 2010)

Phylogenetic group	Num.OTUs	Oxic layer[b] (0–30 m)	Oxic-anoxic transition	Anoxic layer (50–100 m)
Bacteria				
Actinobacteria	1	+	+	+
Bacteroidetes	3	+	+	–
Betaproteobacteria	2	+	+	+
Firmicutes/Clostridium	2	+	–	+
Nitrospira	1	+	+	–
Deltaproteobacteria	1	–	–	+
Mollicutes	1	–	–	+
Chlorobi	1	–	–	+
Archaea[a]				
Euryarchaeota				
Methanosaeta	4	+	+	+
Methanocella	1	–	+	–
Thermoplasmata	1	–	–	+
DHVE-5	1	–	–	+
Thaumarchaeota				
Marine 1.1a	1	+	+	–
Soil 1.1b	11	+	+	+
Crenarchaeota				
C3	2	–	+	+
MCG	6	–	+	+
tMCG	1	–	–	+

[a]*DHVE-5* Deep Hydrothermal Vent Euryarchaeota Group 5, *Marine 1.1a* Marine Crenarchaeota Group 1.1a, *Soil 1.1b* Soil Crenarchaeota Group 1.1b, *C3* Crenarchaeota Group 1.2 or C3, *MCG* Miscellaneous Crenarchaeotic Group or Crenarchaeota Group 1.3, *tMCG* Terrestrial Miscellaneous Crenarchaeotic Group

[b]+, presence; –, absence

as also evidenced for the archaeal assemblage (Llirós et al. 2010). Some phylogenetic groups were commonly retrieved over the whole water column, i.e. *Actinobacteria* and *Betaproteobacteria*, whereas some other groups were exclusively retrieved from the oxic water layer, i.e. *Bacteroidetes*, *Nitrospira* and one *Firmicutes/Clostridium* related sequence, or exclusively detected in anoxic waters, i.e. *Deltaproteobacteria*, *Mollicutes* and *Chlorobi*. Whereas most of the recovered bacterial phylotypes are ubiquitous members of planktonic communities of freshwater environments (*Actinobacteria*, *Bacteroidetes* and *Betaproteobacteria* among others), other phyla (e.g. *Chlorobi*, *Clostridium* and *Deltaproteobacteria*) have been less commonly retrieved in lakes (Newton et al. 2011). It is worth noting that no *Alphaproteobacteria* or *Verrucomicrobia* phylotypes were recovered despite the oligotrophic nature of the lake (Newton et al. 2011). Among the retrieved bacterial OTUs, it is important to notice the detection of *Nitrospira* and *Chlorobi* related sequences. The detection after DGGE band sequencing of one OTU related to *Nitrospira*, a nitrite-oxidizing

bacterium, added putative new players to the N cycle in Lake Kivu. In turn, sequences affiliated to *Chlorobi* and mainly related to *Chlorobium limicola*, a Green Sulfur Bacterium (GSB), were recovered in the main basin from those depths where light reaches anoxic waters and reduced-sulfur compounds were present, but also where bacteriochlorophyll peaks were detected (see Sect. 6.4 below).

6.4 Prokaryotic Cell Abundances, Biomass and Production

To date, only few studies reported abundance and production of picoplankton in East African Great lakes (Pirlot et al. 2005; Sarmento et al. 2008; Stenuite et al. 2009a, b). Sarmento et al. (2008) reported the first data on bacterial abundances in Lake Kivu. Since then, complementary data have been collected covering bacterial abundances and biomass (Malherbe 2008; Nzavuga Izere 2008), heterotrophic bacteria production and bacterial carbon demand (Nzavuga Izere 2008), and finally extracellular release of organic matter by phytoplankton and bacterial re-assimilation (Morana 2009).

During these studies, abundances of prokaryotic cells were measured by flow cytometry. Typical cytograms from Lake Kivu mixolimnion exhibited two main heterotrophic bacteria subpopulations (Fig. 6.4): high nucleic-acid bacteria (HNA) and low nucleic-acid bacteria (LNA). These subpopulations are often present in various aquatic systems (see e.g. Bouvier et al. 2007). The general pattern that emerges from the literature is that HNA cells appear to be not only larger cells but also more active cells, with high specific metabolism and growth, and that changes in total bacterial abundance are often linked to changes in this fraction (Lebaron et al. 2001, but see Jochem et al. 2004; Bouvier et al. 2007 for other possible scenarios on this topic).

HNA were typically more abundant than LNA in the euphotic layer of Lake Kivu, whereas the proportion of LNA increased with depth. The total abundance of prokaryotic cells was between 0.5×10^6 and 2.0×10^6 cells mL^{-1}, with depth maxima located at the 0–20 m layer (Fig. 6.5). HNA abundance in the euphotic layer was positively correlated to chlorophyll *a* concentration, agreeing with a similar pattern reported by Sarmento et al. (2008).

Several subpopulations of heterotrophic bacteria, other than the HNA and LNA, were also observed in cytograms from anoxic waters. They presented different light side scatter and fluorescence (nucleic acid staining) than LNA and HNA subpopulations from surface waters (Fig. 6.4). Further investigations coupling cell sorting and molecular analyses (e.g. Zubkov et al. 2001; Schattenhofer et al. 2011) would be required to identify these microorganisms.

Photosynthetic picoplankton cells were also commonly observed using flow cytometry (Fig. 6.4). In surface samples, the abundance of phycoerythrin-rich picocyanobacteria, identified as *Synechococcus* spp. (Sarmento et al. 2007), ranged between 0.5×10^5 and 2.0×10^5 cells mL^{-1} (Sarmento et al. 2008) and were similar to those observed in Lake Tanganyika (Stenuite et al. 2009a). Vertical depth profiles

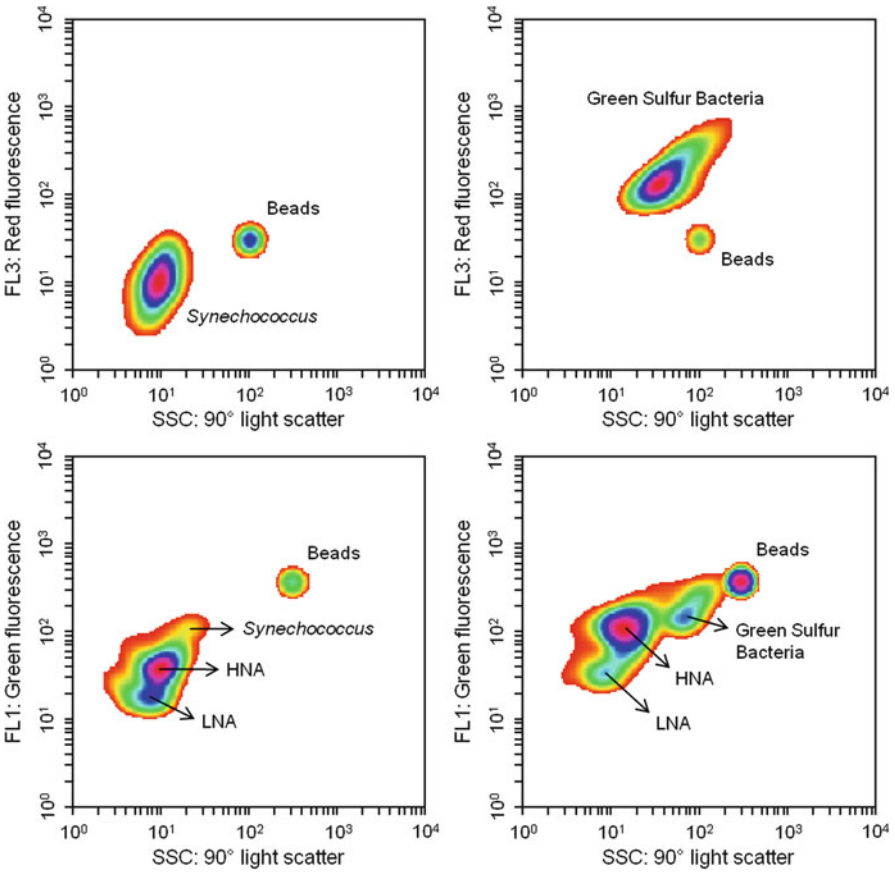

Fig. 6.4 Example of cytograms showing picoplankton cells with natural fluorescence (upper panels) and cells stained with a nucleic acid marker (bottom panels) from Lake Kivu surface waters (5 m depth, left panels) and anoxic waters of the mixolimnion (35 m depth, right panels). The red fluorescence (FL3) is produced by chlorophyll-containing cells. Green Sulfur Bacteria can be distinguished from *Synechococcus* cells because they present a higher red fluorescence signal per cell basis. The nucleic acid stain used (SYBRgreen) develops a green fluorescence (FL1). HNA: bacteria with high nucleic-acid content; LNA: bacteria with low nucleic-acid content

of picoplankton abundances revealed higher values in the euphotic zone than in the deeper mixolimnion, with a sharp decrease at around 30 m depth.

Using *in situ* fluorometry, a permanent chlorophyll peak was detected just below the oxycline (ca. 11 m depth) in Kabuno Bay (Fig. 6.6). A less important chlorophyll peak was also sporadically observed during the rainy season in the upper anoxic layer of the mixolimnion of the main basin (Fig. 6.6). High-performance liquid chromatography pigment analyses of samples collected at the chlorophyll peak allowed the identification of Bacteriochlorophyll *e* (BChl *e*) and isorenieratene, the representative biomarkers for brown-coloured taxa of GSB. No carotenoids from

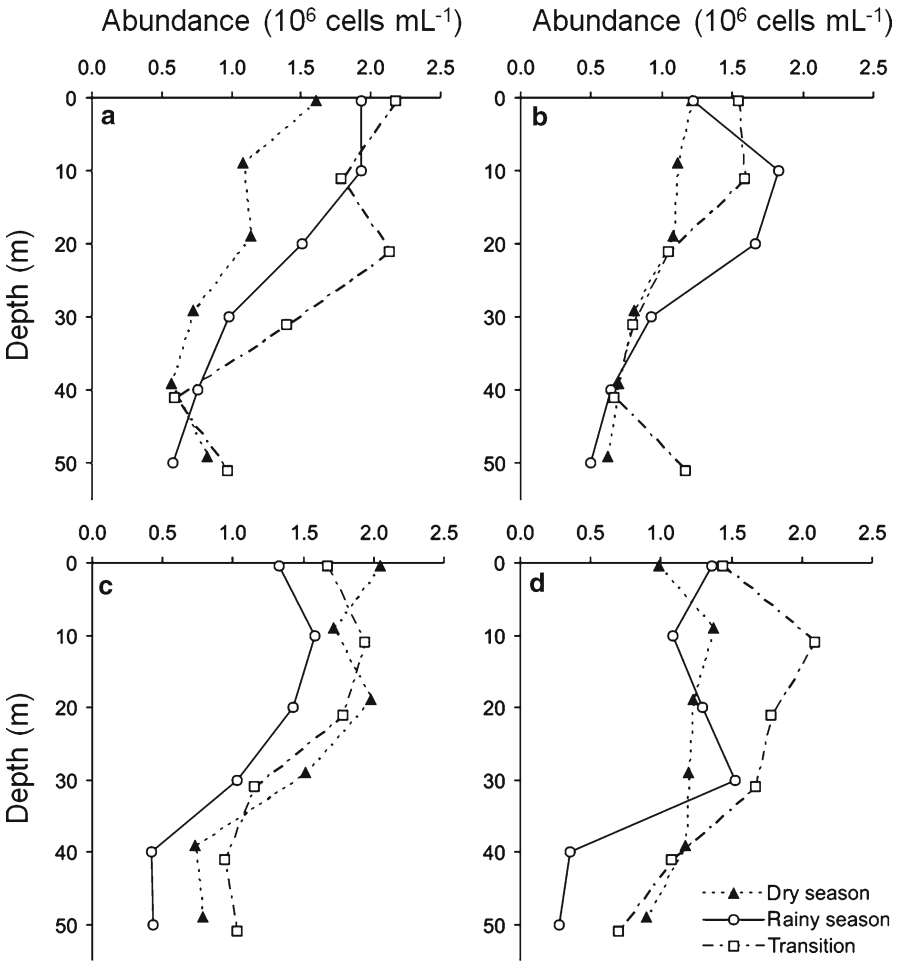

Fig. 6.5 Vertical depth profiles of total abundance of prokaryotic cells observed by flow cytometry, at different seasons, in the Ishungu basin (**a**), in the Kalehe basin (**b**), in the main basin off Kibuye (**c**) and in the main basin off Goma (**d**) of Lake Kivu (modified from Sarmento et al. 2008)

Purple Sulfur Bacteria were detected. These chlorophyll-containing microorganisms were also identified by flow cytometry (Fig. 6.4). GSB are obligatory anaerobic photoautotrophic bacteria, using H_2S, hydrogen or Fe^{2+} as an electron donor (Overmann 2006; Imhoff and Thiel 2010; Table 6.1). They are known to be adapted to extreme low-light conditions (Overmann et al. 1992), such as those prevailing in the lower mixolimnion of the main basin and in Kabuno Bay. In fact, the composition of the main farnesyl-esterified BChl *e* homologs from the population thriving in Lake Kivu suggests a severe *in situ* light limitation (Borrego and García-Gil 1995; Borrego et al. 1997) which deserves further investigation.

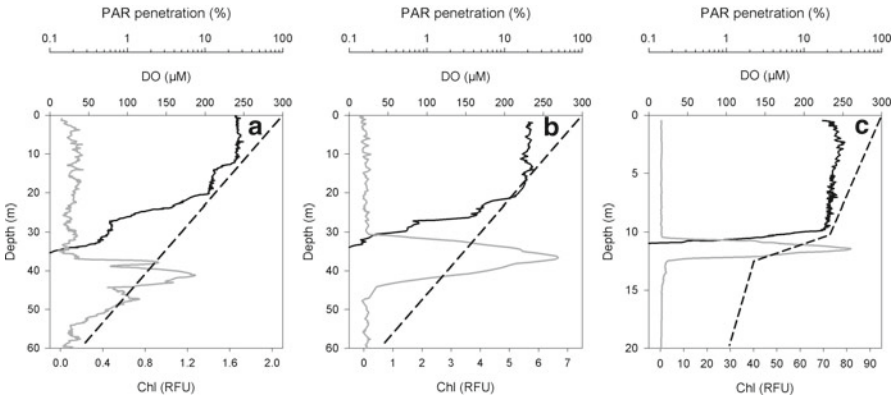

Fig. 6.6 Vertical depth profiles of dissolved oxygen (DO, µM, black line), *in situ* chlorophyll fluorescence (Chl, Relative Fluorescence Unit – RFU, grey line) and photosynthetically active radiation penetration (PAR, %, dashed line) in Lake Kivu, in the Ishungu Basin (**a**), off Gisenyi (**b**) and in Kabuno Bay (**c**) on May 2009

6.5 Bacterial Production and the Phytoplankton-Bacterioplankton Coupling

Several estimates of planktonic bacterial production (BP) were recently performed using ^{3}H-thymidine uptake (Fuhrman and Azam 1980) following the protocol and conversion factors of Stenuite et al. (2009b) described for Lake Tanganyika. Some results are shown in Fig. 6.7. In 2008, the mean BP in the mixed layer off Kibuye was 336 mg C m^{-2} day^{-1} and ranged between 34 and 902 mg C m^{-2} day^{-1} (n = 10, Nzavuga Izere 2008). This range was similar to that of Lake Tanganyika (Stenuite et al. 2009b). In Lake Kivu, BP was relatively low during the dry season, when the mixed layer was deep (Fig. 6.7d, e and f). The highest BP was observed at the beginning of the rainy season, when the mixolimnion started to re-stratify and when the mixed layer was shallow (Fig. 6.7g and h). This dynamics followed that of phytoplankton biomass (Nzavuga Izere 2008).

Considering a bacterial growth efficiency (BGE) of 0.3 (del Giorgio and Cole 1998), the mean bacterial carbon demand (BCD) is expected to be ca. 1120 mg C m^{-2} day^{-1}. The particulate phytoplankton production (PPP) in Lake Kivu is estimated to be around 500–600 mg C m^{-2} day^{-1} (Chap. 5) and is thus lower than the mean BCD. Nevertheless in planktonic systems a variable fraction of total phytoplankton production (TPP) is actually released and directly re-assimilated by bacteria (Baines and Pace 1991; Nagata 2000). This fraction, called the dissolved primary production (DPP), was not taken into account in the initial ^{14}C-incorporation experiments conducted in Lake Kivu and is therefore not accounted for in the PP estimation (Chap. 5). Consequently, additional experiments were conducted for evaluating the percentage of extracellular release (PER) of dissolved organic carbon

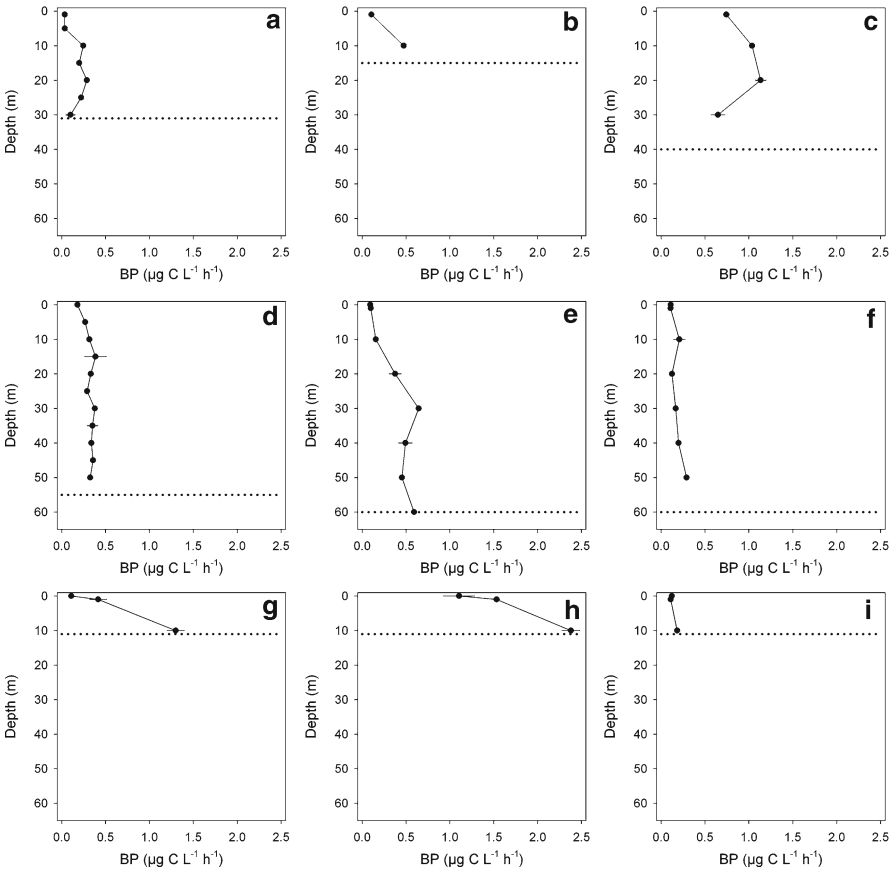

Fig. 6.7 Examples of vertical depth profiles of bacterial production (BP, µg C L^{-1} h^{-1}) off Kibuye in 2008 (**a**, February 14th; **b**, April 29th; **c**, June 3rd; **d**, July 11th; **e**, July 22th; **f**, August 5th; **g**, August 19th; **h**, September 2nd; **i**, September 18th). The dotted lines indicate the lower end of the mixed layer. (data from Nzavuga Izere 2008)

by phytoplankton, i.e. the contribution of DPP to TPP. These experiments, which used a protocol based on ^{14}C uptake kinetics (Morán et al. 2001), were conducted in the Ishungu basin, off Kibuye (main basin) and in Kabuno Bay in May 2009 (Morana 2009). PER was near 50% of total primary production, providing evidence that a substantial fraction of phytoplankton production was excreted. These estimates are in the upper end of the range of commonly observed values for other environments (Baines and Pace 1991; Nagata 2000) but consistent with the high temperature, irradiance and nutrient conditions of this tropical oligotrophic lake (Zlotnik and Dubinsky 1989; Myklestadt 2000; Hansell and Carlson 2002). So, the mean TPP (sum of PPP and DPP) is estimated around 1000–1200 mg C m^{-2} day^{-1} and is in good agreement with the observed BCD, allowing to envision a direct and important transfer of organic matter from phytoplankton to bacterioplankton in Lake Kivu.

6.6 The Assemblage of Small Eukaryotes

The protistan assemblage of Lake Kivu, with the exception of the photosynthetic organisms treated in Chap. 5, is poorly known. For instance, no reliable data on the abundance of phagotrophic protists have been collected so far, whereas a substantial contribution of these microorganisms to the pelagic food web could be envisaged (Tarbe et al. 2011). A recent biodiversity study of the small eukaryotes (0.2–5 μm size fraction) in the surface waters of Lake Kivu, using 18S rRNA fingerprinting, provided data for comparison of the small eukaryotes assemblage with that of Lake Tanganyika (Tarbe 2010).

Two clone libraries were constructed from two different epilimnetic water layers sampled during the rainy season of 2008 (Tarbe 2010). Clone sequences revealed that various phylogenetic groups composed the small eukaryote assemblage in Lake Kivu, including heterotrophs but also photosynthetic microorganisms. Overall, six classes dominated the diversity and represented 78.6% of the retrieved diversity (87.3% of the clones) in the two pooled samples: *Stramenopiles* (21.4%), *Alveolata* (21.4%), *Cryptophyta* (14.3%), *Chytridiomycota* (8.9%), *Kinetoplastea* (7.1%) and *Choanoflagellida* (5.4%). No clones affiliated to *Chlorophyta*, a group poorly developed in Lake Kivu (Chap. 5), were detected from the two Lake Kivu libraries. With closest cultured match rather distant from Lake Kivu sequences, except for some *Chrysophyceae* and *Ciliophora* sequences, the small-eukaryote diversity of Lake Kivu appeared to be poorly represented in culture collections. For instance, Lake Kivu *Kinetoplastea* and *Choanoflagellida* chiefly consisted of new sequences. Moreover, the small eukaryotes assemblage present in Lake Kivu was rather specific, since less than 11% of retrieved sequences were also retrieved in Lake Tanganyika (Tarbe 2010).

6.7 Synthesis and Perspectives

The current data on the microbial community structure in the water column of Lake Kivu are scarce and only based upon very few snapshot studies. Because of the extremely complex vertical structure of this system, which creates totally different ecological niches sometimes within a few centimetres, the microbial diversity is potentially high. High-throughput sequencing technologies will certainly provide a way to access this biodiversity in the near future.

A central role of microbes in the functioning of the Lake Kivu ecosystem can already be envisaged from the available data. The strong temporal coupling between phytoplankton biomass and bacterial abundance and the fact that bacterial carbon demand can be sustained by phytoplankton primary production suggest a preferential transfer of organic matter through the microbial food web in Lake Kivu (Descy and Sarmento 2008). The pivotal role of the microbial food web was recently demonstrated in Lake Tanganyika (Tarbe et al. 2011), where photosynthetic

picoplankton dominated autotrophic biomass and production (Stenuite et al. 2009a, b). Picophytoplankton production and transfer to upper trophic levels should nevertheless be evaluated in Lake Kivu.

Microbial communities developing in the anoxic water compartment carry out different microbial processes from those functioning in the oxic water layers. The production of chemolithotrophs and anoxygenic photoautotrophs (GSB) should be evaluated and compared with the production of oxygenic photoautotrophs (Casamayor et al. 2008). The importance of methanotrophy as a source of energy and carbon for the pelagic food web of Lake Kivu should also be investigated (Jones and Grey 2011).

A promising field of future investigation remains the assessment of the relative role of bacterial and archaeal planktonic assemblages in some important biogeochemical processes, such as nitrification, denitrification and anaerobic methane oxidation. GSB, which are regularly found in the upper anoxic water layers of the lake, also deserve attention, not only as producers but also as sulfide detoxifiers. In this regard, the presence and activity of other bacterial groups involved in sulfur and sulfide oxidation (e.g. *Gamma-* and *Epsilonproteobacteria*, Glaubitz et al. 2009) in oxic/anoxic interfaces of stratified aquatic environments might constitute an interesting topic to be addressed to clarify the contribution of these communities to carbon fixation in sulfide-rich environments.

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