Production of dissolved organic matter by phytoplankton and its uptake by heterotrophic prokaryotes in large tropical lakes

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Abstract

In pelagic ecosystems, phytoplankton extracellular release can extensively subsidize the heterotrophic prokaryotic carbon demand. Time-course experiments were carried out to quantify primary production, phytoplankton excretion, and the microbial uptake of freshly released dissolved organic carbon (DOC) derived from phytoplankton extracellular release (DOCp) in four large tropical lakes distributed along a productivity gradient: Kivu, Edward, Albert, and Victoria. The contributions of the major heterotrophic bacterial groups to the uptake of DOCp was also analyzed in Lake Kivu, using microautoradiography coupled to catalyzed reporter deposition fluorescent in situ hybridization. The percentage of extracellular release (PER) varied across the productivity gradient, with higher values at low productivity. Furthermore, PER was significantly related to high light and low phosphate concentrations in the mixed layer and was comparatively higher in oligotrophic tropical lakes than in their temperate counterparts. Both observations suggest that environmental factors play a key role in the control of phytoplankton excretion. Standing stocks of DOCp were small and generally contributed less than 1% to the total DOC because it was rapidly assimilated by prokaryotes. In other words, there was a tight coupling between the production and the heterotrophic consumption of DOCp. None of the major phylogenetic bacterial groups that were investigated differed in their ability to take up DOCp, in contrast with earlier results reported for standard labeled single-molecule substrates (leucine, glucose, adenosine triphosphate). It supports the idea that the metabolic ability to use DOCp is widespread among heterotrophic prokaryotes. Overall, these results highlight the importance of carbon transfer between phytoplankton and bacterioplankton in large African lakes.

In aquatic systems, the dissolved organic carbon (DOC) pool is a mixture of molecules in a continuum of biological lability, with components from different origins: allochthonous, in freshwaters mainly deriving from the watershed runoff, and autochthonous material produced in situ, such as DOC derived from phytoplankton extracellular release (DOCp) or cell lysis (Myklestad 2000). Both carbon (C) sources can be important to sustain the growth of heterotrophic prokaryotes, but bacteria are highly selective toward the substrate they use (Sarmento and Gasol 2012). In most aquatic systems, heterotrophic bacteria preferentially use labile freshly produced DOCp over more recalcitrant allochthonous compounds (Pérez and Sommaruga 2006). Positive correlations between particulate primary production (pPP) and bacterial production (BP) have been reported for many aquatic systems (Cole et al. 1988; Fouilland and Mostajir 2010). These observations have been used to demonstrate the dependence of heterotrophic bacteria on phytoplankton activity mediated by the release of DOCp. But both phytoplankton and bacterial populations might also be regulated by the same environmental factors, such as inorganic nutrient availability, and then might co-vary with no major interaction (Fouilland and Mostajir 2010). One way to test the interaction strength between primary producers and

bacteria is to measure the phytoplankton production of DOCp (dissolved primary production; dPP) and the kinetics of its uptake by heterotrophic prokaryotes (Morán et al. 2001; Sarmento and Gasol 2012).

Despite its relevance in ecosystem studies, measurements of dPP are scarce, especially in freshwater ecosystems. Based on a literature review, Baines and Pace (1991) proposed an average cross-system percentage of extracellular release (PER) of 13% of total C fixation. However, when comparing freshwater and marine data, it was observed that PER in marine systems was constant across the productivity gradient; whereas, in temperate freshwaters, PER was inversely related to productivity (Baines and Pace 1991). A similar inverse relation in temperate freshwater was found in the recent literature review by Fouilland and Mostajir (2010). There is an ongoing debate about whether DOCp release is an overflow mechanism, whereby DOCp is actively released by healthy phytoplankton cells and is, therefore, constrained by the availability of photosynthates (Fogg 1983; Baines and Pace 1991; Morán and Estrada 2002), or whether DOCp release is a purely passive physiological mechanism, directly proportional to phytoplankton biomass (Bjørnsen 1988; Marañón et al. 2004). Several environmental factors affect PER rates, such as nutrient availability (Obernosterer and Herndl 1995), light conditions (Fogg 1983), and temperature (Zlotnik and Dubinsky 1989); but no consensus has been achieved so far

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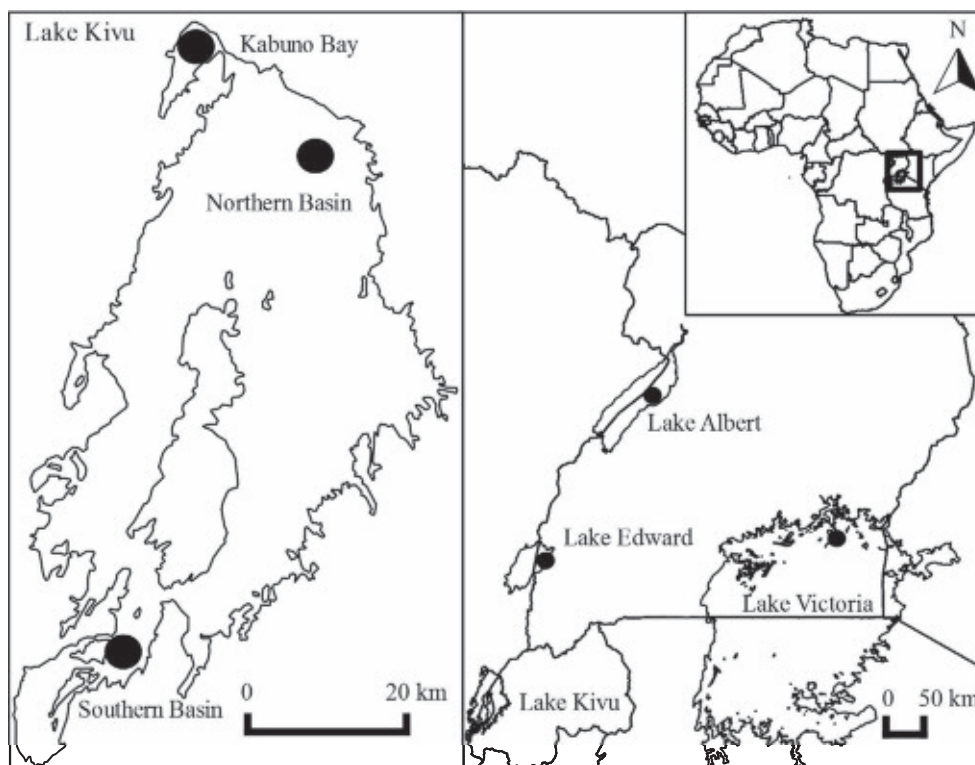* Corresponding author: cedric.morana@ees.kuleuven.be

Fig. 1. Map of lakes Kivu, Albert, Edward, and Victoria, showing the location of the sampling sites (black circle) in April 2009, October 2010, and June 2011 in Lake Kivu, and in May 2012 in lakes Edward, Albert, and Victoria.

on the key environmental factors that determine the importance of PER and on how PER varies in the function of each one of these factors.

The aims of this study were to quantify dPP and the subsequent microbial uptake of DOCp in several large tropical lakes and to elucidate whether the PER was constant, or not, across a range of productivity. Due to the constant exposure to low-nutrient and high-light conditions over long periods of time in oligotrophic tropical lakes, we expected a strong coupling between phytoplankton and heterotrophic prokaryotes through high DOCp release. We performed several time-course experiments of radiocarbon incorporation into dissolved and particulate organic pools, which allowed for correction for heterotrophic uptake of DOCp during the experiments. Furthermore, in one of our study sites (Lake Kivu), we applied the microautoradiography coupled with catalyzed reporter deposition fluorescence in situ hybridization (MAR-FISH) technique to assess the extent to which different groups of heterotrophic prokaryotes were active in the uptake of $DO^{14}Cp$ released by the natural phytoplankton communities and of ^{3}H-leucine, a widely used tracer for BP measurements (Kirchman et al. 1985).

Methods

Study site and water sampling—Data were obtained in large East African lakes Kivu, Edward, Albert, and Victoria (Fig. 1). Sampling in Lake Kivu was conducted in the main lake (northern basin 01°43′S, 29°14′E; southern basin 02°20′S, 28°58′E) and in Kabuno Bay (01°37′S, 29°02′E) in April 2009 (late rainy season), October 2010 (rainy season), and June 2011 (dry season; Fig. 1). Both systems are meromictic but differ in terms of morphometry, with a shallower permanent chemocline in Kabuno Bay compared to the main lake (Borges et al. 2011). Lakes Edward (00°12′N, 29°49′E), Albert (01°48′N, 31°16′E), and Victoria (00°33′N, 33°16′E) were sampled in May 2012. They are shallower than Lake Kivu and are holomictic; consequently, chlorophyll *a* (Chl *a*) concentrations are usually higher in their mixed layer. General limnological characteristics of the four lakes are provided in Table 1.

Sampling procedure—The mixed-layer depth was determined at each occasion, based on in situ observed vertical profiles of temperature and oxygen obtained with a Yellow Springs Instruments 6600 v2 multiparameter probe. Water was collected with a 7 liter Niskin bottle (Hydro-Bios) at a depth interval of 5 m from the surface to the bottom of the mixed layer and was then pooled to obtain a representative sample of the mixed layer. The vertical light attenuation coefficient, K (m^{-1}), was calculated from simultaneous measurements of surface irradiance with a Li-Cor LI-190 quantum sensor and underwater photosynthetically active radiation (PAR) measurements with a submersible Li-Cor LI-193SA spherical quantum sensor. K was derived from the slope of the semi-logarithmic regression between relative

Table 1. General limnological characteristics of the lakes investigated.

	Lake Kivu (main lake)	Lake Kivu (Kabuno Bay)	Lake Edward	Lake Albert	Lake Victoria
Lake area (km²)	2322	48	2325	5300	68800
Catchment area (km²)	4613	3	15840	17000	195000
Maximum depth (m)	485	120	117	58	79
Mean depth (m)	245	65	40	25	40
Mixing regime	Meromictic	Meromictic	Holomictic	Holomictic	Holomictic
Mixed layer temperature (°C)	23.0–24.5*	22.5–24.5*	25.2–27.2†	27.4–29.0†	25.2–27.7‡
Mean Chl *a* (mg m⁻³)	2.2§	2.6*	5–10|	13–60‡	26.5¶
Mean TP concentration (μmol L⁻¹)	0.6*	1.0*	1.4|	2.5|	2.5¶
Mean euphotic depth 1% (m)	18§	10*	13|	6–12‡	9‡

* This study.
† Verbeke 1957.
‡ R. Mugidde unpubl.
§ Darchambeau et al. 2014.
\| Lehman et al. 1998.
¶ Guildford and Hecky 2000.

quantum irradiance and depth. The mean irradiance in the mixed layer (I_{Zm}) was calculated following Riley (1957):

$$I_{Zm} = I_0 \times (1 - e^{-K \times Zm})/(K \times Zm)$$

where I_0 is the incident irradiance at the surface (μmol photon m⁻² s⁻¹), K (m⁻¹) is the vertical light attenuation coefficient, and Zm (m) is the mixed-layer depth.

Chemical analyses—Phosphate (PO_4^{3-}) concentrations were quantified spectrophotometrically following standard procedures (American Public Health Association 1998). Measurements of pH were carried out with a Metrohm (6.0253.100) combined electrode calibrated with U.S. National Bureau of Standards buffers of pH 4.002 (25°C) and pH 6.881 (25°C), prepared according to Frankignoulle and Borges (2001). Measurements of total alkalinity (TA) were carried out by open-cell titration with HCl 0.1 mol L⁻¹ on 50 mL water samples, and data were quality checked with Certified Reference Material acquired from Andrew Dickson (Scripps Institution of Oceanography, University of California, San Diego). Typical precision for TA measurements was better than ± 3 μmol L⁻¹. Dissolved inorganic carbon (DIC) was computed from pH and TA measurements, using the carbonic acid dissociation constants of Millero et al. (2006). Water samples for DOC concentrations were filtered through preflushed 0.2 μm syringe filters in 40 mL borosilicate vials with Teflon-coated screw caps and were preserved with 0.1 mL of H_3PO_4 (50%). DOC concentrations were measured with a customized Thermo Hipertoc coupled to a Delta+XL isotope ratio mass spectrometer, whereby complete oxidation of the sample is ensured by a combination of sodium persulfate addition, heating, and ultraviolet radiation. Quantification and calibration was performed with a standard solution of sucrose purchased from the International Atomic Energy Agency (IAEA-C6). Typical reproducibility for DOC analyses was on the order of < 5%.

Chl a concentration—Chl *a* concentrations were determined by high-performance liquid chromatography (HPLC). At each sampling site, 3 liters of water from the mixed layer pool was filtered on a Macherey-Nägel GF-5 filter (nominal porosity of 0.4 μm). Pigment extraction was carried out in 10 mL of 90% HPLC grade acetone. After two sonication steps of 15 min separated by an overnight period at 4°C, the extracts were stored in 2 mL amber vials at −25°C. HPLC analysis was performed following the method described in Sarmento et al. (2008), with a Waters system comprising a photodiode array and fluorescence detectors. Calibration was made using commercial external standards (DHI Lab Products). Precision for Chl *a* measurement was better than ± 7%.

pPP and dPP and heterotrophic uptake of freshly excreted compounds—pPP and microbial uptake are usually estimated by tracing the incorporation of a radioactive tracer (¹⁴C) into two different size fractions representing phytoplankton (> 2 μm) and heterotrophic prokaryotes (< 2 μm; Cole et al. 1982). However, the important contribution of picophytoplankton (< 2 μm) to total phytoplankton biomass in some African lakes (21% in Lake Kivu; Sarmento et al. 2008) complicates the physical separation of the primary producers from heterotrophic prokaryotes by such a size-selective filtration. This problem may be overcome by measurement of the time-course incorporation of ¹⁴C into the dissolved (< 0.2 μm) and particulate (> 0.2 μm) phase, the latter including both heterotrophic prokaryotes and phytoplankton (Morán et al. 2001; Morán and Estrada 2002). Results are then modeled by a compartmental organic C exchange model, extensively described by Morán and Estrada (2002), and are briefly summarized in the next section. Due to the importance of picophytoplankton in some lakes studied, this approach was preferred for this study.

In the field, aliquots of 40 mL of water from the mixed-layer pool were introduced in transparent 70 mL sterile polycarbonate cell culture flasks. Each bottle was spiked with 62.5 μCi of NaH¹⁴CO₃ (specific activity of 40–60 mCi mmol⁻¹; Perkin Elmer) and then was incubated at in situ temperature under a constant PAR of 200 μmol photon m⁻² s⁻¹ provided by a Philips fluorescent lamp (PL

Table 2. Mixed-layer depth (Z_m; m), mean daylight irradiance in the mixed layer (I_{Zm}; μmol photon m^{-2} s^{-1}), particulate and dissolved primary production (pPP and dPP; mg C m^{-3} h^{-1}), percentage of extracellular release (PER; %), concentrations of Chl a (mg m^{-3}), dissolved organic carbon (DOC; mg C m^{-3}), freshly excreted dissolved organic carbon (DOCp; mg C m^{-3}), and DOCp turnover times (h) in lakes Kivu (Southern bay, Northern bay, Kabuno Bay), Edward, Albert, and Victoria.

Station	Year	Z_m	I_{Zm}	pPP	dPP	PER	Chl a	DOC	DOCp	Turnover DOCp
Southern bay	2009	20	220	0.8	1.1	57	1.9	1967	1.7	1.5
Southern bay	2010	12.5	381	2.8	2.8	50	2.1	1924	3.6	1.3
Southern bay	2011	30	186	1.7	3.0	64	2.1	1757	3.9	1.3
Northern bay	2009	22.5	249	1.6	2.6	62	1.2	1911	6.4	2.1
Northern bay	2010	22.5	243	4.0	3.9	49	2.3	1872	2.3	0.6
Northern bay	2011	42.5	76	2.9	2.7	48	2.0	1709	3.6	1.3
Kabuno Bay	2009	10	162	3.9	3.9	50	2.4	2341	8.3	2.5
Kabuno Bay	2011	5	265	5.1	3.7	42	2.6	1779	13.2	3.6
Lake Edward	2012	7.5	145	26.8	6.5	20	9.9	4757	5.5	0.8
Lake Albert	2012	20	95	27.4	5.9	18	5.4	4383	3.6	0.6
Lake Victoria	2012	15	108	172.7	9.4	5	5.1	1807	102.3	10.9

55W Daylight DeLuxe). This light intensity was close to the mean I_{Zm} of the investigated lakes at the time of the experiments (Table 2) and to the saturation irradiance value (I_k) reported for Lake Kivu (318 μmol photon m^{-2} s^{-1}; Darchambeau et al. 2014). The incubations lasted for 5–6 h, and, at 30 or 60 min intervals, biological activity was stopped by adding neutral formaldehyde (0.02% final concentration) into two transparent flasks. Two additional flasks covered with aluminum foil were processed in the same manner immediately at the beginning and at the end of the experiment, providing a dark incorporation control. The bottles were then kept overnight at 4°C before subsequent processing. From each flask, a 20 mL subsample was filtered under low-pressure vacuum on membrane filters (Millipore GSWP; 0.22 μm nominal porosity) to separate the particulate from the dissolved fraction. In order to remove the labeled inorganic C (DI^{14}C), liquid samples (DO^{14}C) were acidified with 1 mL of HCl (6 mol L^{-1}) and were left open overnight in an orbital shaker, and filters (PO^{14}C) were fumed overnight with concentrated HCl. Radioactivity of the filters and liquid samples was measured using a Packard Tri-Carb Liquid Scintillation Counter with Ultima Gold (Perkin Elmer) as scintillation cocktail and the external standard method for quench correction. T_0 values were subtracted from values of subsequent samples in order to correct for the abiotic radiocarbon incorporation. The radioactivity values in dark incorporation control bottles at the end of every experiment were never significantly different than values in the respective T_0-bottles; therefore, they were not subtracted.

Organic C exchange model—Assuming steady state, the experimental results were fitted with a three-compartment model of organic C exchange, extensively described in Morán et al. (2001) and Morán and Estrada (2002). The rate of change of the radiocarbon content in the three different compartments is described by the following equations (Fig. 2):

$$dC_1/dt = -k_{(2,1)} \times C_1 + k_{(1,2)} \times C_2 - k_{(3,1)} \times C_1 \quad (1)$$

$$dC_2/dt = k_{(2,1)} \times C_1 - k_{(1,2)} \times C_2 + k_{(2,3)} \times C_3 \quad (2)$$

$$dC_3/dt = k_{(3,1)} \times C_1 - k_{(2,3)} \times C_3 \quad (3)$$

where C_1 is the radiocarbon concentration in the DIC pool, C_2 is the radiocarbon concentration in the particulate organic carbon (POC) pool, and C_3 is the radiocarbon concentration in the DOC pool; $k_{(i,j)}$ is the rate constant of C flux from pool j to pool i (in h^{-1}). Hence, $k_{(2,1)}$ is the rate constant of pPP, $k_{(1,2)}$ is the rate constant of respiration of synthesized POC, inferred from its influence on the PO^{14}C kinetics, $k_{(3,1)}$ is the rate constant of phytoplankton excretion or dPP, and $k_{(2,3)}$ is the rate constant of heterotrophic uptake of freshly produced DOCp.

A striking particularity of the model lies in the origin of the DOCp production flux. Although it is obvious that DOCp should first be incorporated by phytoplankton before its excretion, the model considers the DOCp to be directly produced from a subcompartment integrated into the DIC pool, the organic C fated for release (OCR) pool. This subcompartment is defined as an intracellular
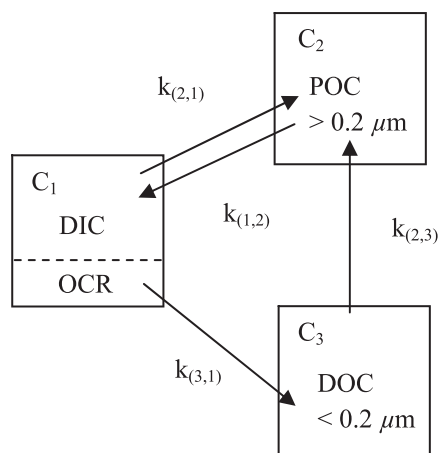

Fig. 2. Schematic representation of the three-compartment model applied on the experimental data. DIC = dissolved inorganic carbon, DOC = dissolved organic carbon, POC = particulate organic carbon, OCR = intracellular pool of organic carbon fated for release pool; *see* text and Morán et al. (2001) for further details.

phytoplankton C pool of organic compounds and is assumed to be quasi-instantaneously in isotopic equilibrium with the DIC pool (Morán et al. 2001; Morán and Estrada 2002). This assumption was confirmed experimentally, for example, by the absence of any lag phase in DO^{14}C kinetic curve when phytoplankton incorporates labeled-DIC (Wiebe and Smith 1977; our own results below).

Least-squares nonlinear fitting of the model to dissolved (DO^{14}C) and particulate (PO^{14}C) radioactivity measurement was performed using Saam II software (Epsilon Group). Data were weighted by the inverse of the standard deviation of duplicates. The pPP and dPP rates were respectively calculated from $k_{(2,1)}$ and $k_{(3,1)}$ multiplied by the DIC concentration. PER (%) was calculated as:

$$PER = dPP/(pPP + dPP) \times 100 \qquad (4)$$

MAR-FISH—MAR-FISH allows tracking at the single-cell level of substrate uptake by heterotrophic organisms. We investigated the heterotrophic uptake of two types of substrate: ^{3}H-leucine, a widely used tracer for bulk BP measurements (Kirchman et al. 1985), and an uncharacterized mixture of DO^{14}Cp produced by natural lake water phytoplankton assemblages during the experiment. In 2011, in parallel to the dPP experiments carried out in Kabuno Bay and in the southern basin of Lake Kivu, four supplementary culture flasks were filled with 20 mL of water from the mixed layer pool and were spiked with 62.5 μCi of NaH^{14}CO$_3$. Two of these flasks were incubated for 5 h under a constant PAR as described above, whereas the two others were covered with aluminum foil and were incubated in the dark, in order to correct for bacterial DI^{14}C uptake. The isotopic equilibrium between the DIC and DOCp pool was reached before the end of the 5 h incubations, and the specific activity of the DO^{14}Cp was 258 μCi mmol^{-1}. Finally, two additional culture flasks were spiked with ^{3}H-leucine (0.5 nmol L^{-1} final concentration; specific activity 162 Ci mmol^{-1}) and were kept in the dark for 5 h. Incubations were stopped by adding neutral formaldehyde (1.8% final concentration), and the samples were stored overnight at 4°C, followed by gentle filtration of 5 mL on a 0.2 μm isopore membrane polycarbonate filter (Millipore). Filters were then stored at −20°C until further processing.

Catalyzed reporter fluorescent in situ hybridization (CARD-FISH) analyses were carried out as follows. The cells were permeabilized with lyzozyme and achromopeptidase prior to the hybridization. Several horseradish peroxidase (HRP)-probes were used to characterize the composition of the bacterial community in the water samples, using the procedure described by Alonso-Sáez and Gasol (2007). The HRP-labeled probes used were EUB338-II -III (targets most Eubacteria), GAM42a (targets most Gammaproteobacteria), BET42a (targets most Betaproteobacteria), ALF968 (targets most Alphaproteobacteria), and CF319 (targets many groups belonging to Bacteroidetes). The unlabeled competitors BET42a for GAM42a and GAM42a for BET42a were used. Specific hybridization conditions were established by adding formamide to the hybridization buffer (45% formamide for ALF968, 55% for the other probes). All probes were purchased from Biomers. Counterstaining of CARD-FISH preparations was done with 4,6 diamidino-2-phenylindole (DAPI; final concentration 1 μg mL^{-1}). Between 500 and 1000 DAPI-positive cells were counted with an Olympus BX61 epifluorescence microscope in a minimum of 10 fields.

MAR-FISH analyses were performed after hybridization following the CARD-FISH protocol. The filters were glued onto glass slides with an epoxy adhesive (Uhu plus). The slides were embedded in 46°C tempered photographic emulsion (Kodak NTB-2) containing 0.1% agarose (gel strength 1%, > 1 kg cm^{-2}) and were placed in a dark room on an ice-cold metal bar for about 5 min to allow the emulsion to solidify. They were subsequently placed inside black boxes at 4°C until development. Based on preliminary trials, we used an optimal exposure time of 7 d for samples incubated with ^{3}H-leucine and 10 d for samples incubated with DO^{14}Cp. For development, exposed slides were submerged for 3 min in a developer (Kodak D-19), rinsed 30 s with distilled water, and then placed for 3 min in a fixer (Kodak T-max), followed by 5 min of washing with tap water. The slides were then dried in a dessicator overnight, stained with DAPI (1 μg mL^{-1}), and inspected under an Olympus BX61 epifluorescence microscope. CARD-FISH positive cells (hybridized with the specific probe) appear in bright green under blue light excitation. Additionally, MAR-FISH positive cells contain dark silver grains accumulated above the bacterial cells on the radiographic emulsion, resulting from radioactive decay of labeled substrates.

Results

dPP and pPP and bacterial uptake of DOCp—The kinetics of incorporation of labeled substrate in the POC and DOC pools followed two distinct patterns (Fig. 3a,b). In all experiments, the radioactivity increased in the POC pool almost linearly with time, whereas the increase of radioactivity in the DOC pool was initially linear but rapidly leveled off at a maximum value after 2–4 h.

The measured pPP rates in Lake Kivu ranged from 0.8 mg C m^{-3} h^{-1} to 5.1 mg C m^{-3} h^{-1}, and the dPP rates from 1.1 mg C m^{-3} h^{-1} to 3.9 mg C m^{-3} h^{-1} (Table 2). Normalized to Chl *a*, the total primary production (dPP + pPP) in Lake Kivu averaged 2.8 ± 0.8 mg C mg Chl a^{-1} h^{-1}. PER values ranged between 42% and 64%, with an average of 53%. Higher rates of pPP and dPP, but lower PER values, were found in lakes Edward, Albert, and Victoria (Table 2). Volumetric rates of pPP and dPP were positively correlated with Chl *a* concentration (Table 3; Fig. 4a,b). Furthermore, dPP was positively correlated to pPP, and the slopes of the model I and model II linear regressions were both significantly lower than 1 (Table 3; Fig. 5), implying that PER decreased with increasing primary production rates.

The turnover time of the DOCp in surface waters, calculated as the inverse of the $k_{(2,3)}$ constant rate, ranged between 0.6 h and 3.6 h, with an average of 1.8 ± 0.9 h in Lake Kivu. The turnover time of DOCp was in the same
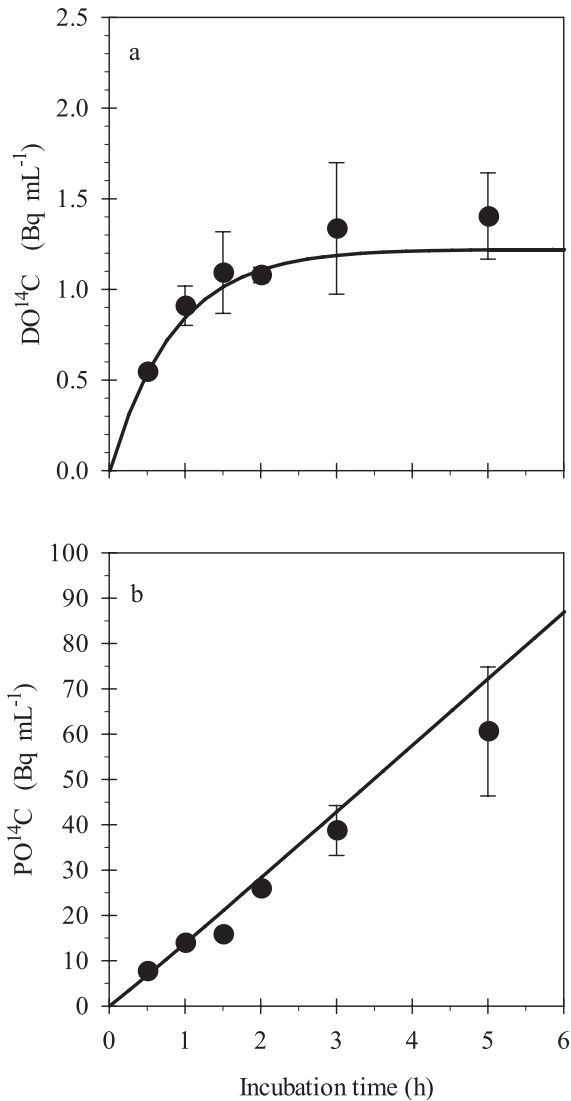
Fig. 3. Example of the kinetics of phytoplankton production of (a) $DO^{14}C$ and (b) $PO^{14}C$ during 5 h of incubation in Lake Edward. The fitted curves are derived from the three-compartment model presented in Fig. 2. Symbols are mean of duplicate measurements, and error bars are maximum and minimum values.
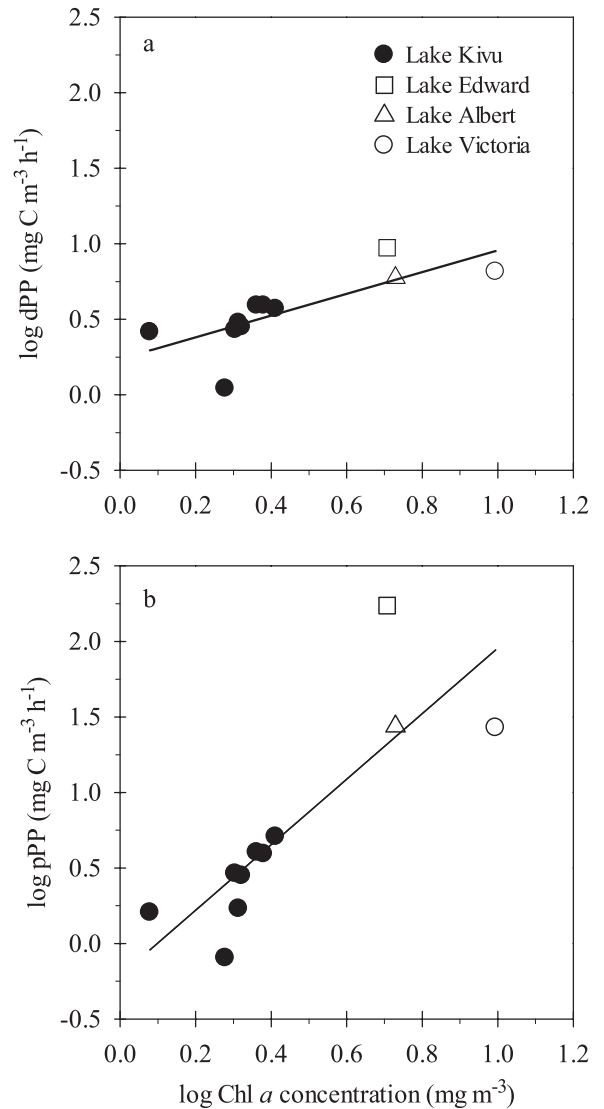
Fig. 4. Relationships between log(Chl *a*) and (a) log(dPP) or (b) log(pPP) in lakes Kivu, Edward, Albert, and Victoria. Continuous lines illustrate model I linear regression lines.

range in lakes Edward (0.8 h) and Albert (0.6 h) but was clearly higher in the eutrophic Lake Victoria (10.9 h; Table 2). The size of the DOCp pool, calculated as the dPP rate multiplied by the turnover time of DOCp, ranged between 1.7 mg C m^{-3} and 13.2 mg C m^{-3} (but with a higher value of 102.3 mg C m^{-3} for Lake Victoria), and contributed less than 1% to the DOC pool in lakes Kivu,

Edward, and Albert, and approximately 5% in Lake Victoria (Table 2).

3H-leucine and DO14Cp uptake by different bacterial phylogenetic groups—The bacterial community structure and the percentage of active cells taking up ^{3}H-leucine and DO^{14}Cp were investigated in 2011 in the southern basin of Lake Kivu and in Kabuno Bay, where, respectively, 75%

Table 3. Summary of model I and model II log–log linear regressions between particulate and dissolved primary production (respectively, pPP and dPP) and between pPP and Chl *a*. In parentheses, 95% confidence interval.

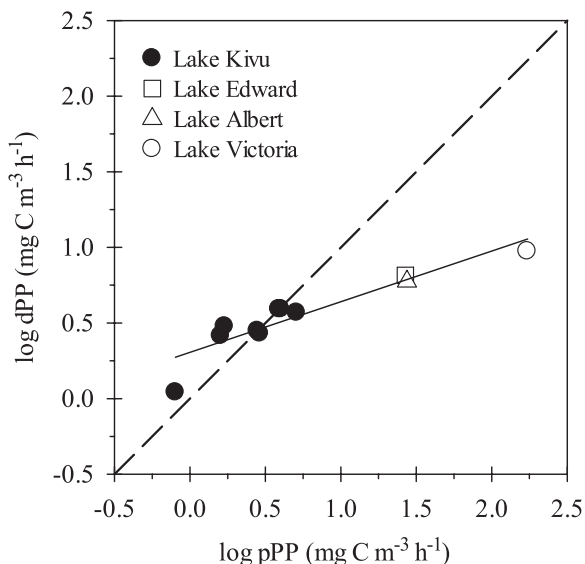|			Model I regression				Model II regression
y	*x*	*n*	Slope	Intercept	R^2	*p*	Slope
log(pPP)	log(Chl *a*)	11	2.16 (1.05, 3.27)	−0.21 (−0.77, 0.35)	0.68	<0.05	2.10 (1.79, 2.41)
log(dPP)	log(Chl *a*)	11	0.07 (0.01, 0.12)	0.37 (0.11, 0.56)	0.46	<0.05	0.60 (0.38, 0.83)
log(dPP)	log(pPP)	11	0.33 (0.23, 0.44)	0.31 (0.23, 0.44)	0.85	<0.0001	0.33 (0.25, 0.40)

Fig. 5. Relationship between log(pPP) and log(dPP) in lakes Kivu, Edward, Albert, and Victoria. Continuous line illustrates model I linear regression line; dashed line is the 1:1 line.

Fig. 6. Relative abundances of heterotrophic prokaryotes analyzed by CARD-FISH and scaled to total DAPI counts in Lake Kivu, in June 2011. "Other EUB" stands for cells identified with the probe mix EUB338-II-III but not detected with any of the probes used, targeting the large phylogenetic groups of Eubacteria: Alpha, Beta, Gammaproteobacteria, and Bacteroidetes. "No EUB" stands for cells counted with DAPI but not identified with the probe mix EUB338-II-III.

and 83% of total cells counts were hybridized with EUB338-II-III probe (EUB, Eubacteria cells; Fig. 6). Members of Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, and Bacteroidetes jointly comprised 49% and 50% of the total cell count and covered 65% and 60% of the bacterial domain in the southern basin and Kabuno Bay, respectively. Bacterial community structure, at the large group level targeted by CARD-FISH probes, was fairly similar between the two sites (Fig. 6). After 5 h of incubation in the dark with ^{3}H-leucine, 21% and 17% of the Eubacteria cells were found to take up ^{3}H-leucine in the southern basin and Kabuno Bay, respectively. Of the Eubacteria cells, 12% and 11% were labeled at the end of the incubation when considering DO^{14}Cp uptake. In the two stations, we found that less than 1% of the EUB338 cells were labeled by DI^{14}C; therefore, bacterial DIC fixation via chemoautotrophic or anapleurotic pathways seemed to have been insignificant in the surface waters of Lake Kivu during these short-term incubations.

By comparing the relative contribution of each bacterial group in substrate uptake with its relative abundance (Fig. 7a,b), we can examine whether these broad bacterial groups participated in the DO^{14}Cp and ^{3}H-leucine uptake proportionally to their contribution to community structure. The ^{3}H-leucine uptake pattern was roughly similar between the two stations. In the southern basin and at Kabuno Bay station, we found that 85% and 75% of all the bacteria taking up ^{3}H-leucine belonged to two groups: Betaproteobacteria and Alphaproteobacteria. Accounting together for 42% and 41% of the bacterial community abundance, the contribution of these two groups to ^{3}H-leucine uptake was clearly higher than expected from their relative abundance. By contrast, the Bacteroidetes, the Gammaproteobacteria, and the bacterial cells unrelated to any of the bacterial groups investigated in this study
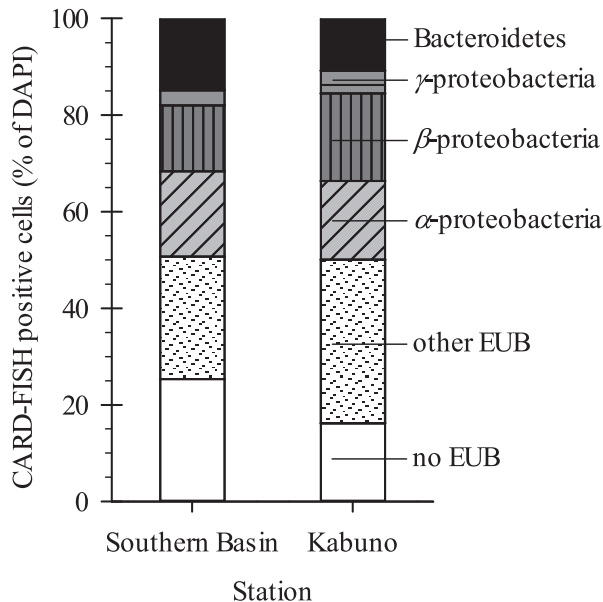
(other EUB), were underrepresented in ^{3}H-leucine uptake (Fig. 7a). The pattern of DO^{14}Cp uptake was different compared to that of ^{3}H-leucine. All the data points were closer to the 1:1 line, indicating that the bacterial groups were participating in DO^{14}Cp uptake proportionally to their relative contribution to the bacterial community. For instance, Betaproteobacteria and Alphaproteobacteria, the two dominant and overrepresented groups in the portion of the assemblage consuming ^{3}H-leucine, accounted for only 47% and 41% of cells taking up DO^{14}Cp in the southern basin and Kabuno Bay, respectively. Because they contributed 42% and 41% to the bacterial community structure, their share in DO^{14}Cp consumption was proportional to their in situ abundance.

Discussion

In this study, we gathered a first consistent set of concurrent measurements of pPP and dPP rates in tropical African lakes. The methodology to measure dPP differs largely among different studies available in the literature, and only a few of them account for the heterotrophic uptake of labeled DOCp during the course of the experiment, which can lead to an underestimation of PER. A way to overcome this bias is to observe the kinetics of labeling in the DOC and POC pools and to fit the data with a compartmental organic C exchange model (Morán et al. 2001; Morán and Estrada 2002).

It has recently been proposed that high DOC release rates, especially in oligotrophic systems, are not sustained by active excretion of healthy phytoplankton, but rather by
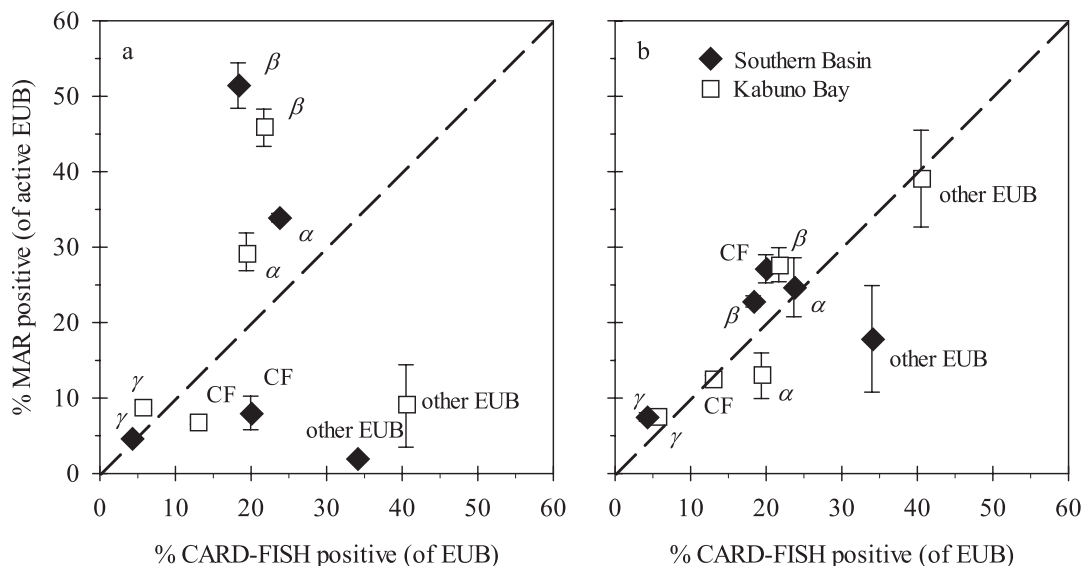
Fig. 7. Relative contribution of each phylogenetic group to the Eubacteria (EUB) community (CARD-FISH positive) against their relative contribution to the (a) ^{3}H-leucine or (b) DO[14]Cp uptake (MAR positive) in Lake Kivu, in June 2011. Symbols are mean of duplicate measurements, and error bars are the maximum and minimum values. The dashed line is the 1 : 1 line. α = Alphaproteobacteria, β = Betaproteobacteria, γ = Gammaproteobacteria, CF = Bacteroidetes, other EUB = cells identified with the probe mix EUB338-II-III but not detected with any of the other probes used.

the lysis of dying cells (Agustí and Duarte 2013). During our short-term experiments, a significant amount of labeled DOCp was released after only 30 min of incubation, and no lag phase was observed in the appearance of radioactivity in the DOC pool. According to Lancelot (1979), such a lag phase is expected when the specific activity of the intracellular pool of molecules that can be exchanged with the external medium does not immediately reach a stable value. Therefore, the absence of lag phase (Fig. 3a) implies that the excreted compounds originate from a small intracellular pool that has a relatively high turnover rate (Marañón et al. 2004). Based on this observation, it appears unlikely that cell lysis or trophic processes such as zooplankton grazing were the main mechanisms of DOCp release, because then a large amount of non-recent, presumably unlabeled metabolites would have been part of the intracellular pool of molecules fated for release, considerably increasing its size. Instead, it seems to be driven by a purely physiological mechanism of phytoplankton excretion of freshly produced photosynthates (Marañón et al. 2004).

Why do phytoplankton cells actively release photosynthates? Previous studies have shown that high irradiance (Zlotnik and Dubinsky 1989) and nutrient limitation enhance phytoplankton excretion, particularly in P-limited situations (Obernosterer and Herndl 1995; Myklestad 2000). Under high-light conditions, such as in the tropics, photorespiration, the fixation of O_2 on ribulose-1,5-phosphate catalyzed by ribulose-1,5-bisphosphate carboxylase oxygenase (RubisCO) with glycolate as a byproduct, is an effective protection mechanism against photoinhibition. It keeps the consumption of nicotinamide adenine dinucleotide phosphate and adenosine triphosphate (ATP) at high levels and lowers the saturation of the electron transport chain, preventing the production of reactive

oxygen species. An important fraction of this glycolate can be actively excreted by phytoplankton cells to the water (Fogg 1983), where it can be rapidly consumed by heterotrophic prokaryotes (Lau et al. 2007). Besides photorespiration, the active release of photosynthate by phytoplankton cells can be viewed as a mechanism occurring under nutrient limitation, when the synthesis of molecules containing N or P is not possible (Wood and Van Valen 1990; Morán et al. 2002). Because of the nutrient limitation, a fraction of the freshly fixed C is a surplus and is, therefore, excreted. It has also been proposed that DOCp release could be a purely passive diffusion process of molecules across the membrane due to a concentration gradient and, thus, directly proportional to phytoplankton biomass (Bjørnsen 1988; Marañón et al. 2004).

The PER value measured in Lake Kivu (42–64%) was much higher than the cross-system average of 13% proposed by Baines and Pace (1991) but was within the large range of values reported for lakes (3–82%). PER decreased with increasing pPP, the highest value being observed in the oligotrophic Lake Kivu and the lowest in the eutrophic Lake Victoria (Fig. 8). The use of a multiple linear regression model combining log-transformed I_{Zm} and PO_4^{3-} to predict PER explained a high amount of the variance (adjusted r^2 = 0.51) and shows that both independent variables had a significant effect on PER (PER = $-92.13 + 49.99 \times \log I_{Zm} - 27.16 \times \log PO_4^{3-}$; p = 0.030 for log I_{Zm}; p = 0.036 for log PO_4^{3-}; n = 11). No significant correlations were found when using a univariate linear model ($p > 0.05$) to predict PER. Hence, high I_{Zm} and low PO_4^{3-} concentration in the mixed layer had a significant combined positive effect on phytoplankton excretion. Overall, these results support the view that environmental factors play an important role in the control of PER in large African lakes. Furthermore, the relationship
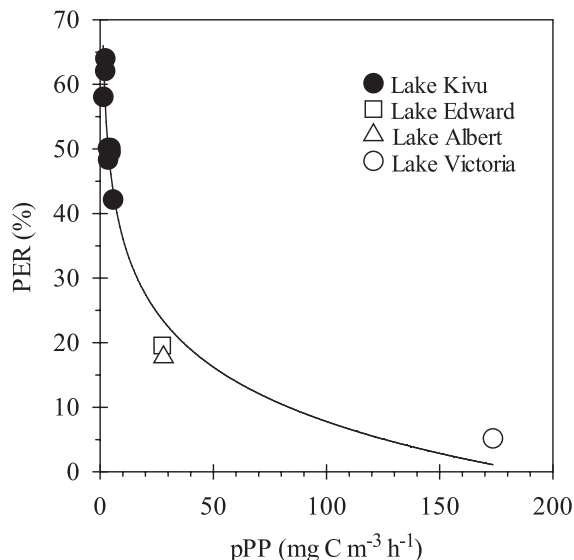
Fig. 8. Relationship between the percentage of extracellular release (PER) and particulate primary production (pPP) in the four lakes studied.

between PER and pPP was nonlinear, because PER tended to level off at a minimum value in the most productive waters. Because the debate around the physiological mechanisms driving phytoplankton excretion remains unresolved (Fogg 1983; Bjørnsen 1988), we hypothesize that the passive diffusion and the transport-mediated mechanisms are not mutually exclusive, but that the dominance of one on the other may change. For instance, we would suggest that passive DOCp leakage across the membrane would be relatively constant, whereas active loss by a transport-mediated mechanism would be related to environmental conditions, such as nutrient and light availability. Hence, in tropical oligotrophic waters, under high-light and low-nutrient conditions, the transport-mediated mechanism could be the main process responsible for extracellular excretion, but its dominance over the passive diffusion mechanism would decrease along the productivity gradient.

In a review of published dPP values corrected for heterotrophic uptake, Fouilland and Mostajir (2010) found a similar nonlinear inverse relationship between PER and primary production in freshwater temperate lakes (Fig. 9), but the slope of the log–log relationship between dPP and pPP in the African tropical lakes (0.23–0.44, 95% confidence interval) is significantly lower than the one for temperate lakes (0.48–0.65, 95% confidence interval; ANCOVA, $f_{1,81} = 8.047$; $p < 0.01$). This difference indicates that, in unproductive waters, PER is higher in tropical lakes than in their temperate counterparts, consistent with the higher and relatively constant irradiance distinctive of tropical environments. We predict, therefore, that high PER values should be expected in other large oligotrophic tropical lakes, such as Lake Malawi and Lake Tanganyika.

The mean annual BP in the mixed layer of Lake Kivu was 336 mg C m^{-2} d^{-1} ($n = 10$ sampling dates in 2008; Llirós et al. 2012). Bacterial respiration has not been directly measured in Lake Kivu; but, using a range of
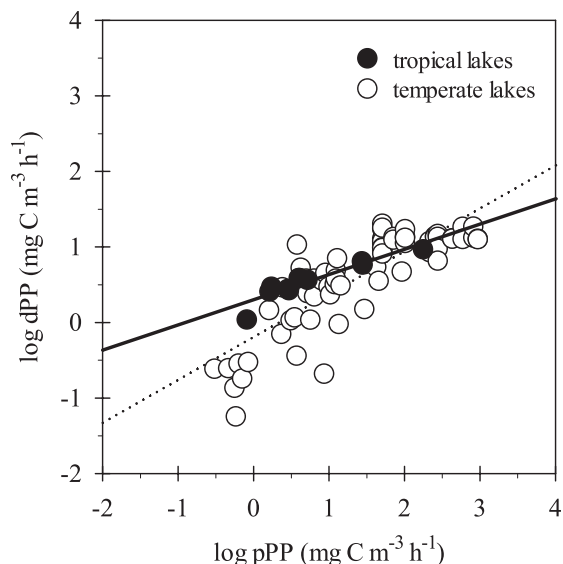

Fig. 9. Relationship between log(pPP) and log(dPP) in several African large tropical lakes (solid line, model I regression, our dataset) and several temperate lakes (dotted line, model I regression, data from Fouilland and Mostajir 2010). The tropical lakes regression is described in Table 3, and the temperate lakes regression is log(dPP) = -0.57 log(pPP) $- 0.19$; $R^2 = 0.71$, $p < 0.0001$; $n = 73$.

bacterial growth efficiency (BGE) values expected in tropical systems (10–20%), the bacterial C demand (BCD; BCD = BP/BGE) would range between 1680 mg C m^{-2} d^{-1} and 3360 mg C m^{-2} d^{-1}. The mean phytoplankton pPP in Lake Kivu (620 mg C m^{-2} d^{-1}; Darchambeau et al. 2014) is clearly unable to support the BCD by itself, but the high PER values (42–64%) reported in this study would allow total phytoplankton production to meet 32–103% of the BCD in Lake Kivu. This reasoning can be extended to Lake Tanganyika, where total primary production would sustain 36–117% of BCD (Table 4). The range of BCD estimates that can be sustained by total phytoplankton production is wide, but it strongly depends on the choice of BGE values. In addition, other processes that were not measured during this study, such as grazing by zooplankton or cell lysis, can significantly contribute to the release of autochthonous DOC.

DOCp is a highly complex pool of diverse molecules whose exact chemical natures are unknown. Carbohydrates of various sizes should be predominant in the excreted products (Myklestad 2000). It has recently been shown that the nature of the DOCp depends on phytoplankton community composition (Sarmento et al. 2013). In the large African lakes investigated, the DOCp standing stock was relatively small compared to the high dPP rates observed, and the turnover times of the DOCp were, therefore, short (Table 2). In other words, the consumption of DOCp was tightly coupled to its production, and only a small amount of DOCp accumulated in the water. This suggests that the DOCp pool was mainly composed of labile molecules that were preferentially assimilated by heterotrophic prokaryotes over other organic C sources. These observations highlight the importance of a direct

Table 4. Estimates of bacterial carbon demand (BCD) using a range of bacterial growth efficiencies (BGE) and bacterial production (BP) data obtained from the literature. Estimates of the fraction of the BCD met by phytoplankton production (PP = dPP + pPP) using the range of PER measured in Lake Kivu during this study.

Variable	Lake Kivu	Lake Tanganyika
BCD (mg C m^{-2} d^{-1})	1680–3360	1550–3100
BGE (%)	10–20	10–20
BP (mg C m^{-2} d^{-1})	336*	310†
pPP (mg C m^{-2} d^{-1})	620‡	654†
PER (%)	42–64§	
PP/BCD	0.32–1.03	0.36–1.17

* Llirós et al. 2012.
† Sténuite et al. 2009.
‡ Darchambeau et al. 2014.
§ This study.

transfer of organic matter from phytoplankton to bacterioplankton in Lake Kivu.

The fraction of cells active in the uptake of leucine after 5 h of incubation (average 19%) was close to the range of values of metabolically active cells reported in the literature (20–40%, del Giorgio and Gasol 2008), but the fraction of active cells in DOCp uptake (average 11.5%) was low compared to this range. However, these studies used low-molecular-weight, very labile molecules as tracers, but the DOCp pool presumably comprises higher-molecular-weight substrates that require more complex enzymatic pathways and that are, therefore, harder to assimilate in short incubations. Additionally, the activity of some light-sensitive heterotrophic prokaryotes might have been inhibited by the high light irradiance during our incubations. Also, the labeled DO^{14}Cp was diluted with nonlabeled and equally labile compounds, which decreased the sensitivity of the technique at this range of incubation times.

In contrast to earlier studies that used standard labeled molecules, such as leucine, ATP, or glucose (Alonso-Sáez and Gasol 2007), our MAR-FISH results show that all different phylogenetic groups of heterotrophic prokaryotes were involved in the uptake of molecules belonging to the uncharacterized pool of DO^{14}Cp. The high abundance and activity of members of the Betaproteobacteria group is not surprising; their abundance has been found to be positively related with the amount of algal-derived substrates (Simek et al. 2008; Paver et al. 2012), and they are usually ubiquitous in freshwaters. The relatively high abundance of Alphaproteobacteria and Bacteroidetes members is more difficult to interpret. Members of these groups are generally not abundant in freshwaters but seem to be effectively resistant to eukaryote predation (Newton et al. 2011); however, bacterivory pressure has never been estimated in Lake Kivu. Overall, our observations support the idea that the metabolic ability to process labile algal-derived dissolved organic matter is widespread among broad prokaryote groups (Landa et al. 2013). Furthermore, it is possible that the diversity of molecules excreted by the phytoplankton community present in Lake Kivu can support a variety of ecological niches for heterotrophic prokaryotes, thereby allowing them to avoid direct resource competition. It was, indeed, recently shown that the composition of the extracellular release of dissolved free amino acids by phytoplankton was species dependent (Sarmento et al. 2013).

^{3}H-leucine is a widely used tracer for BP measurements (Kirchman et al. 1985). This method requires a parallel estimate of the leucine-to-C conversion factor, defined as the yield of bacterial biomass per unit of leucine incorporated. Nevertheless, a difference in ability to take up leucine among different phylogenetic groups of prokaryotes has been reported (Pérez et al. 2010), and community composition could affect leucine-to-C conversion factor estimates (Alonso-Sáez et al. 2010). To our best knowledge, our study is the first to compare the uptake pattern of ^{3}H-leucine to the uptake pattern of DO^{14}Cp produced by a natural phytoplankton community. In Lake Kivu, members of Alphaproteobacteria, Betaproteobacteria, and Bacteroidetes were involved in DO^{14}Cp uptake proportionally to their abundance, but they differed strongly in their ability to take up ^{3}H-leucine. This discrepancy suggests that leucine may not be a good representative of other naturally occurring, but more complex, organic C sources, such as DOCp.

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