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Phosphorus enrichment and carbon depletion contribute to high *Microcystis* biomass and microcystin concentrations in Ugandan lakes

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Abstract

We investigated the factors influencing cyanobacterial biomass and microcystin (MC) concentrations in several Ugandan lakes from September 2008 to February 2009. We characterized thermal structure, light availability, nutrient concentrations, chlorophyll *a*, phytoplankton $\delta^{13}C$ (as an indicator of CO_2 limitation), and phytoplankton community composition and abundance as well as MC concentrations. We used these data to test several hypotheses based on previous research in temperate lakes regarding the factors that encourage high cyanobacterial biomass and MC concentrations. Site characteristics that appeared to favor high cyanobacterial biomass (especially *Microcystis*) included: high total phosphorus concentrations, low total nitrogen to total phosphorus (TN : TP) ratios, and possibly low CO_2 availability. Light availability, total nitrogen concentrations, and thermal structure of the water column were not related to cyanobacterial biomass. MC concentrations were strongly related to *Microcystis* biomass (and were not related to the biomass of any other cyanobacterial taxa), which was positively correlated with total phosphorus and chlorophyll *a* concentrations. MC cell content may be moderated by CO_2 availability, with MC cell quotas tending to be lower where the potential for C-limitation of photosynthesis was higher. In these phosphorus-rich tropical lakes, the shallowest study sites were most conducive to the development of large standing crops of *Microcystis* and high MC concentrations. The environmental conditions that appear to favor high cyanobacterial biomass and MC concentrations in our Ugandan study lakes are similar to what has been observed for temperate lakes.

There is a well-documented relationship between the occurrence of harmful cyanobacterial blooms and anthropogenic input of nutrients to freshwater ecosystems (Paerl and Fulton 2006). Also, climate warming is expected to lead to shifts in phytoplankton community composition that favor bloom-forming cyanobacteria that are often capable of toxin production (Paerl and Huisman 2009). Cyanobacterial toxins commonly produced in freshwater include the hepatotoxic cyclic peptide microcystins (Sivonen and Jones 1999), which can be produced by several taxa, including *Microcystis*, *Anabaena*, *Anabaenopsis*, *Planktothrix* (*Oscillatoria*), and *Nostoc* (Sivonen and Jones 1999).

Microcystin (MC) concentrations depend on both the abundance of cyanobacteria belonging to genotypes capable of MC production and the amount of MC produced by these cyanobacteria. A number of environmental factors have been evaluated for their effect on MC production, including: light, temperature, nutrient concentrations, nutrient ratios, and pH (Zurawell et al. 2004). The factors driving cellular MC production are complex and often appear to be contradictory; however, there is evidence that synthesis and resultant cellular concentrations may be highest where conditions are favorable for cell growth (Orr and Jones 1998; Briand 2005).

To date, most studies of the factors that influence biomass of MC-producing cyanobacteria and cellular MC concentrations have focused on temperate systems (Zurawell et al. 2004). As a result, considerably less is known about MC dynamics in tropical lakes. In temperate lakes, strong seasonal changes in temperature, light, and mixing mean that high cyanobacterial biomass is often only observed in the summer and early fall (Munawar and Munawar 1986). This seasonality results in variable limnological conditions and nonlinear succession of phytoplankton communities, which complicate determination of fundamental controls on cyanobacterial abundance and MC concentrations. However, in tropical lakes with their "endless summer" (Kilham and Kilham 1990), there is the potential for year-round dominance of potentially toxic cyanobacteria, often at high biomasses (Ganf 1974; Kling et al. 2001). In these lakes, internal cycling and near steady-state conditions can dominate and can simplify the search for controlling factors. We hypothesized that comparative studies in a range of tropical lakes would reveal those factors most critical to determining cyanobacterial abundance and MC concentrations and would allow for comparison with results from studies in temperate lakes.

Freshwater lakes in Uganda, East Africa, are critical sources of both water and fish for large human populations (Mugidde et al. 2003). Presence of MC has been reported for Lakes Victoria and Edward as well as for several smaller Ugandan lakes (Sekadende et al. 2005; Okello et al. 2009; Poste et al. 2011). To assess the biological and environmental controls on MC concentrations in tropical lakes, six Ugandan lakes known to have substantial cyanobacteria populations, including the tropical Great Lakes Victoria and Edward, as well as four smaller Ugandan lakes—Lake George, Lake Mburo, and the crater lakes Saka and Nkuruba (Fig. 1; Table 1)—were

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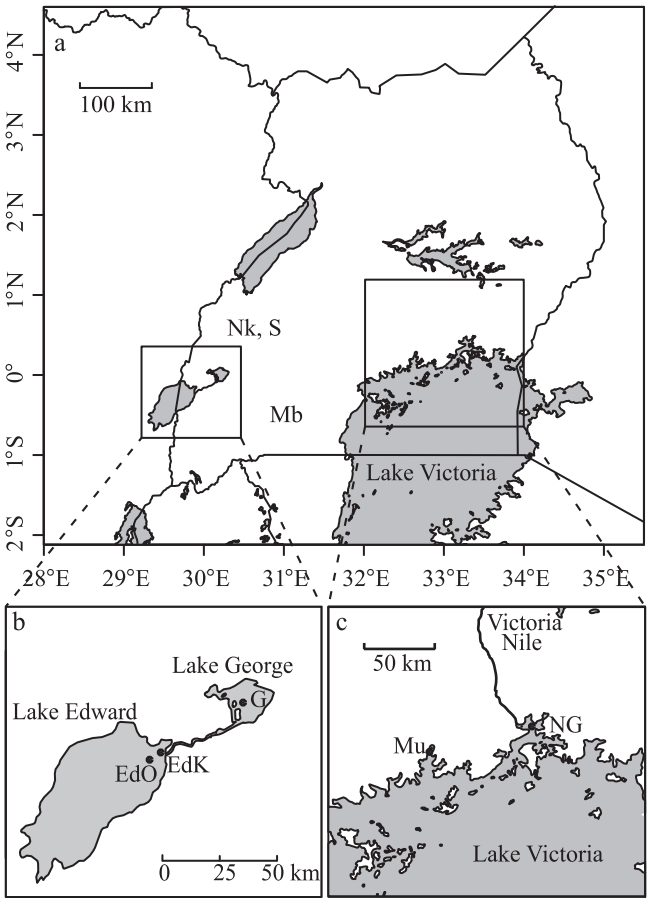

Fig. 1. (a) Map of Uganda showing location of study sites; (b) detailed map of Lakes Edward and George showing the Lake George study site as well as the nearshore and offshore Lake Edward study sites; and (c) detailed map of northern Lake Victoria showing the Napoleon Gulf and Murchison Bay sampling sites. Note that the Victoria Nile (indicated on map) exits Lake Victoria via Napoleon Gulf. Site codes used in maps are given in Table 1.

chosen for a detailed temporal study. MC concentrations, phytoplankton community composition, and physicochemical variables were characterized over a 6-month period when maximal cyanobacterial abundance typically occurs.

This study was designed to test whether the factors that determine cyanobacterial abundance and MC concentrations in tropical lakes are similar to what has been observed in the literature for temperate lakes. Hypothesized factors affecting cyanobacterial abundance that we tested in our study include: water column stability and high water temperature (Paerl and Huisman 2009), high phosphorus (P) and nitrogen (N) concentrations (Trimbee and Prepas 1987; Downing et al. 2001), low N : P ratios (Smith 1983), low soluble reactive silica (SRSi; Schelske and Stoermer 1971), low light availability (Mur 1983), high pH (Shapiro 1997), and low CO_2 (Paerl 1988). We also tested the hypothesis that MC production (as indicated by cell quota of MC) is positively related to cell growth (Orr and Jones 1998). Factors likely to be limiting to cell growth that we examined include light, temperature, P, N, and carbon (C) availability. In this study, we also present a novel way of quantifying CO_2 availability relative to expected photosynthetic CO_2 demand based on phytoplankton stable C isotopic ratios normalized to chlorophyll *a* (Chl *a*) concentrations.

Methods

Study sites—Although cyanobacterial blooms can develop seasonally and usually briefly in the pelagic areas of the African Great Lakes (Hecky and Kling 1987), persistent high cyanobacterial biomass generally develops only in shallower nearshore areas or in shallow lakes where the water column is generally well mixed throughout much of the year and internal nutrient loading from the sediments typically dominates nutrient fluxes to the water column. Consequently, our study sites were chosen to offer a gradient of depths from 2.8 to 33.4 m (Table 1) and were sampled over a 6-month period. Although lake depth was our primary ordination, each of the lakes has other attributes that may influence limnological conditions and cyanobacterial biomass.

Lake Victoria is the world's largest tropical lake by surface area, and the current study includes two embayments in northern Lake Victoria: Murchison Bay and Napoleon Gulf (Fig. 1). Shallow Murchison Bay is located in a densely populated urban area and provides water to and receives sewage outfall from Kampala, Uganda's largest city. Napoleon Gulf is situated 60 km east of Murchison Bay in a mixed urban and agricultural area, and receives wastewater from the city of Jinja. However, due to its location at the outflow of Lake Victoria to the Nile River, Napoleon Gulf is well flushed with water from the offshore regions of the lake.

Lake George is a highly productive shallow lake (mean depth of 2.4 m) that is dominated by cyanobacteria yearround (Ganf 1974). Water from Lake George flows into the larger, deeper, and less productive Lake Edward (which is at nearly the same elevation as Lake George) via the Kazinga Channel. Samples were collected from central Lake George, nearshore Lake Edward (''Edward Kazinga''; at the opening of the Kazinga Channel to the lake), and offshore Lake Edward (''Edward Open''; $\sim$ 2.5 km offshore; Fig. 1). Meanwhile, Lake Mburo lies within Lake Mburo National Park and is an important source of drinking water for wild game and supports a large population of hippopotamuses (*Hippopotamus amphibius*; Nyakoojo and Byarujali 2010).

Lakes Saka and Nkuruba are volcanic crater lakes in Western Uganda. Lake Saka's catchment has been highly affected by deforestation and agriculture, and the lake is hypereutrophic (Campbell et al. 2006). Meanwhile, Lake Nkuruba is a small lake surrounded by an intact rainforest ecosystem; it has a maximum depth of 38 m and a permanently anoxic hypolimnion below 9–15 m (Chapman et al. 1998).

Sample collection and physical observations—Samples were collected on a monthly basis between September 2008 and February 2009 (except for Murchison Bay and

Table 1. General characteristics of Ugandan study lakes and sampling stations. Z_{max} refers to maximum lake depth. Study sites are arranged by depth (shallowest to deepest), and data sources are listed as footnotes.

Study lake	Sampling site	Code	Latitude	Longitude	Elevation (m)	z_{max} (m)	Site depth (m)	Area (km^2)	Volume (km^3)
George*	George	G	0°00′S	30°11′E	913	7	2.8	250	0.5
Saka†	Saka	S	0°41′N	30°15′E	1520	8.5	3.2	0.15	0.00005
Mburo‡	Mburo	Mb	0°39′S	30°56′E	1231	4	3.2	13	0.325
Edward§	Edward Kazinga	EdK	0°13′S	29°53′E	—	—	3.5	—	—
Victoria|	Murchison Bay¶	Mu	0°15′N	32°39′E	—	7	5.2	18	0.113
Edward	Edward Open	EdO	0°13′S	29°52′E	—	—	7.3	—	—
Victoria	Napoleon Gulf#	NG	0°24′N	33°14′E	—	20.5	17.5	26.5	0.22
Nkuruba**	Nkuruba	Nk	0°31′N	30°18′E	1519	38	33.4	0.03	0.00048

* Viner and Smith 1973.
† Melack 1978.
‡ Nyakoojo and Byarujali 2010.
§ Whole-lake information for Lake Edward: elevation of 912 m; z_{max} of 120 m; area of 2325 km^2; and volume of 76.7 km^3 (Lehman 2004).
| Whole-lake information for Lake Victoria: elevation of 1134 m; z_{max} of 75 m; area of 66,368 km^2; and volume of 2598 km^3 (Silsbe 2004).
¶ Haande et al. 2011.
# Jackson 2004.
** Chapman et al. 1998.

Napoleon Gulf, where samples were collected every 2 weeks). Secchi depth (SD) was measured and temperature profiles were taken using a FluoroProbe (bbe-Mondaenke). Euphotic zone water samples were collected using a Niskin sampler from under the water surface, at SD and at twice the SD, and then pooled. In Murchison Bay and Napoleon Gulf, samples were always collected in the morning, whereas at the remaining sites samples were collected in the morning where possible, but occasionally were collected later in the day.

Light attenuation was estimated based on Chl *a* concentrations using the relationship of Silsbe et al. (2006) for Lake Victoria ($k_{PAR} = 0.20(\text{Chl } a)^{0.52}$); this equation was used because Chl *a* concentrations were generally high, and likely accounted for most of the light extinction in these lakes. The correlation ($r = 0.75$, $p < 0.01$, $n = 55$) between inverse SD (SD^{-1}) and Chl *a* confirms that chlorophyll concentrations are a primary driver of water transparency in these lakes. Mean water column irradiance in the mixed layer as a proportion of surface light (Guildford et al. 2000) was calculated using k_{PAR} and mixed depth (based on temperature profiles). Absolute mean mixed-layer irradiance was calculated assuming a surface irradiance of 50,000 mmol photons m^{-2} d^{-1} (from Guildford et al. 2000; representing the amount of light reaching the earth's surface at Lake Victoria's latitude, assuming a clear, cloudless atmosphere).

Nutrient and chlorophyll a *analyses*—Water samples were processed as soon as possible on the same day as sampling. Whole water samples were preserved with 0.0075 v : v 4N H_2SO_4 for analysis of total phosphorus (TP), nitrate (NO_3^-), and total nitrogen (TN). TP samples were digested with persulfate and analyzed as soluble reactive phosphorus (SRP) as in Stainton et al. (1977), whereas NO_3^- and TN samples were analyzed using a Lachat chemical analyzer (Lachat QuikChem® FIA+ Series 8000; QuikChem® Method 31-107-04-1). For analysis of particulate silica (PartSi) and dissolved nutrients, whole water was filtered through a Millipore 0.2 μm polycarbonate filter, and both the filter and filtrate were frozen until analysis. The filtrate was analyzed (within 30 d of sample collection) for ammonium (NH_4^+, using the indophenol method), SRP, and SRSi using the methods outlined in Stainton et al. (1977). PartSi samples were digested using 2 mL of 0.5 mol L^{-1} sodium hydroxide, then neutralized and analyzed as SRSi.

Whole water was filtered through Whatman GF/F filters (nominal pore size of 0.7 μm) and filters were kept frozen until analysis of Chl *a* (measured fluorometrically after acetone extraction; Stainton et al. 1977) or particulate phosphorus (PartP; measured as SRP after persulfate digestion; Stainton et al. 1977). For analysis of particulate carbon (PartC) and nitrogen (PartN), water was filtered through pre-combusted (at 450°C for 4 h) Whatman GF/F filters, and filters were stored frozen until dried (at 60°C for 24 h), then kept in a desiccator until analysis. Analysis was carried out at the University of Waterloo (Ontario, Canada) using an elemental analyzer (Exeter CEC-440 CHN/O/S Elemental Analyzer).

Phytoplankton $\delta^{13}C$ *and* CO_2 *availability*—Phytoplankton samples were collected using vertical net hauls (20 μm mesh), and were filtered onto pre-combusted quartz-fiber filters (Whatman QM-A). Filters were oven-dried at 60°C for 24 h and were not acidified, as the net phytoplankton samples were not expected to contain appreciable amounts of carbonate for these lakes. Stable C ($\delta^{13}C$) isotopic ratios were determined using an elemental analyzer–isotope ratio mass spectrometer (Micromass IsoChrom).

Stable C isotopic ratios ($\delta^{13}C$) of phytoplankton are largely determined by the degree of C isotopic fractionation during photosynthesis as well as the $\delta^{13}C$ value of the substrate for photosynthesis. High phytoplankton biomass and growth rates as well as low CO_2 concentrations can lead to reduced isotopic discrimination during photosynthesis

due to instantaneous C limitation, resulting in higher $\delta^{13}C$ values (Hecky and Hesslein 1995; Hecky et al. 2010); whereas in high-pH and low-CO_2 conditions, many cyanobacterial taxa (including *Microcystis*) can use bicarbonate (HCO_3^-) as an alternative C source (Paerl 1988), leading to even more enriched (higher) $\delta^{13}C$ values (Hecky and Hesslein 1995). As such, phytoplankton $\delta^{13}C$ values can indicate CO_2 availability in the system at the time of sampling, with higher phytoplankton $\delta^{13}C$ values tending to indicate lower CO_2 availability. By normalizing phytoplankton $\delta^{13}C$ to Chl *a* concentrations (which should generally reflect volumetric photosynthetic demand for CO_2), we are able to gain insight into the CO_2 availability relative to expected CO_2 demand, with higher normalized values indicating low availability relative to demand, and a higher likelihood of C limitation of photosynthesis and phytoplankton growth rates. Chlorophyll-normalized phytoplankton $\delta^{13}C$ (‰) values are reported in units of ‰ (log Chl *a*)$^{-1}$, where Chl *a* is chlorophyll *a* concentration in μg L^{-1}.

Phytoplankton community composition—Whole water samples were preserved with Lugol's iodine shortly after collection. Samples were settled in a Ütermohl chamber and phytoplankton were identified and enumerated to the species level using an inverted microscope. Cell volume was calculated based on Wetzel and Likens (1991), and biomass was calculated assuming a cell specific gravity of 1 g cm^{-3}.

MC analysis—MC in water was measured using indirect competitive enzyme-linked immunosorbent assay (ELISA; Abraxis, Microcystins-Adda ELISA kits, product number 520011). This is a congener-independent ELISA based on the detection of the Adda side-chain found in microcystins and nodularins (Fischer et al. 2001). Total MC (cell-bound and dissolved) was measured in whole water, and dissolved MC was measured in filtrate (filtered through Whatman glass-fiber filter with a nominal pore size of 0.7 μm). Whole water samples were prepared for use in ELISA assays through chemical lysis (using the Abraxis QuikLyse method; Loftin et al. 2008). Cell-specific MC concentrations (cell quotas) were calculated as MC concentration divided by number of *Microcystis* spp. cells (as determined through microscopy), and are expressed in units of femtograms MC per cell *Microcystis*.

Statistical analyses—Data for all variables, with the exception of mean water column temperature, station depth, and phytoplankton $\delta^{13}C$ (which were normally distributed), were log-transformed. Pearson correlation coefficients (r) were calculated to test for relationships between the measured physicochemical variables and to assess the influence of environmental factors on cyanobacterial biomass, MC concentrations, and MC cell quota. We recognize that correlations cannot establish causation, but we will argue that correlations provide valuable indication of causal relationships where ecological mechanisms can be proposed to explain such relationships. However, we will also remain conscious of the fact that environmental data are inherently variable, and as such, some factors that could be important determinants of cyanobacterial biomass or MC concentrations may not be identified through linear correlation analysis. All statistical analyses were carried out using R, version 2.11.1 (R Development Core Team 2010).

Results

Phytoplankton community composition—Cyanobacteria dominated the phytoplankton biomass throughout the whole study period in most lakes, and all sites experienced occasions when cyanobacteria made up $>$ 80% of total biomass. Diatoms were also important contributors to the phytoplankton biomass at several of the study sites, particularly at offshore Lake Edward where they made up, on average, 50% of total biomass and dominated the phytoplankton biomass from mid-November until February. The cyanobacterial communities at the study sites primarily consisted of *Microcystis*, *Planktolyngbya*, *Anabaena*, and occasionally *Cylindrospermopsis*. However, in Lake Saka, *Planktothrix* was the dominant cyanobacterial taxon. *Planktolyngbya* spp. was, on average, the largest contributor to the cyanobacterial biomass in Lakes Edward, George, Mburo, Nkuruba, and Napoleon Gulf. *Microcystis* spp. dominated the cyanobacteria in Murchison Bay, and made up an appreciable portion of the total phytoplankton biomass at all sites but Lake Nkuruba.

Physical observations—Temperature and thermal stratification: Mean water column temperatures over the study period were between 24.7°C and 26.6°C for all sites except for the two higher-elevation crater lakes where mean water column temperatures were lower (22.1°C ± 0.5°C in Lake Saka and 22.8°C ± 0.4°C in Lake Nkuruba). With the exception of Lake Nkuruba, where a persistent and well-defined thermocline was observed throughout the whole study period, and Napoleon Gulf where stable thermal stratification (which we define as a thermocline of $>$ 1°C m^{-1}, excluding the top meter of the water column) was observed on several occasions between late September and November, the study sites all had well-mixed water columns when sampled. At many sites, although persistent stable stratification was not observed, the upper 10–50 cm of the water column was often warmer than the underlying waters (by 0.5–3°C, typically observed later in the day), suggesting diurnal stratification.

Light penetration and daily mean light: Mean SD over the study period was highest at the three deepest sampling sites, and ranged from 0.4 to 0.7 m at the remaining shallower sites (Table 2). Mean mixed-layer irradiances (Table 2) ranged from 10–21% of surface irradiance, and were lowest in Napoleon Gulf (minimum: 1.7 mmol photons m^{-2} min^{-1}) and Murchison Bay (minimum: 2.0 mmol photons m^{-2} min^{-1}), where mean mixed-layer irradiance was consistently lower than the level at which light limitation of *Microcystis* growth has been previously observed (4.8 mmol photons m^{-2} min^{-1}; Wiedner et al. 2003).

Nutrient and Chl a *concentrations and correlations*—Nutrient (total and dissolved) and Chl *a* concentrations,

Table 2. Summary of physicochemical observations for the study lakes (using all data, $n = 55$). Sites are arranged by depth (as in Table 1), and results are reported in the format of mean ± standard deviation. Abbreviations are defined in the text, with the exception of: Temp, temperature; Light, mean mixed-layer light intensity, in mmol photons m^{-2} min^{-1}; NH_4^+, ammonium.

Site	Secchi depth (m)	Temp (°C)	Light	Chl *a* (μg L^{-1})	TP (μg L^{-1})	TN (μg L^{-1})	NH_4^+ (μg L^{-1})	SRP (μg L^{-1})	SRSi (μg L^{-1})	PartSi (μg L^{-1})	$\delta^{13}C$ (‰)
George	0.37±0.08	26.4±1.0	5.5±2.8	138.0±39.1	186.5±26.2	1462±1008	5.9±4.0	9.9±4.9	7969±1616	15.0±8.3	−9.6
Saka	0.44±0.11	22.1±0.5	5.6±1.0	90.0±36.3	175.0±32.2	2440±1675	8.5±5.5	24.7±14.7	9046±2091	11.2±5.1	−20.7
Mburo	0.48±0.10	24.7±0.7	7.2±0.8	48.6±10.1	106.8±11.1	1934±964	6.9±4.0	10.5±3.0	6861±426	9.5±1.5	−12.6
Edward Kazinga	0.50±0.25	25.5±2.1	7.9±3.9	66.3±46.2	129.1±54.7	1707±643	5.4±3.8	10.3±6.1	7437±1262	12.5±6.5	−13.0
Murchison Bay	0.72±0.14	25.6±0.5	3.5±1.2	96.5±38.1	100.3±22.5	2108±742	29.3±30.4	7.2±5.2	805±313	12.3±5.1	−14.9
Edward Open	1.05±0.27	26.6±0.5	6.9±4.0	21.3±22.8	58.9±9.2	1013±276	4.8±4.1	10.6±5.2	6040±1232	12.2±3.8	−19.0
Napoleon Gulf	1.40±0.23	25.7±0.7	3.4±2.2	24.7±18.4	60.0±16.2	1644±1022	23.8±41.6	11.5±11.0	757±296	10.4±8.3	−16.5
Nkuruba	1.80±0.39	22.8±0.4	8.6±2.5	6.2±2.2	35.6±8.6	1323±750	6.4±6.5	8.2±3.7	5510±1087	1.1±0.5	−26.7

although generally high, exhibited substantial temporal variability (note standard deviations in Table 2) within study sites (e.g., at Napoleon Gulf, TN concentrations ranged from 530–3600 μg L^{-1} over the study period). Nitrate was rarely detected (detection limit of ~ 16 μg L^{-1}), with measurable concentrations (always below 30 μg L^{-1}) observed on two occasions in Murchison Bay, and one occasion in each of Napoleon Gulf, Lake George, and Edward Kazinga. Physicochemical and biomass data were used to test for significant association ($p < 0.01$) based on Pearson correlation coefficients (Table 3). Across all lakes (including all data; $n = 55$), positive relationships were observed between Chl *a* and TP (Fig. 2a), PartP, PartSi, TN, PartN : PartP, total phytoplankton biomass, and Cyanophyta biomass. Negative relationships were observed between Chl *a* and SD, station depth, mean mixed-layer irradiance, and TN : TP ratio. Station depth was negatively correlated with TP, Chl *a*, and total phytoplankton biomass, and positively correlated with SD (Table 3).

Nutrient deficiencies—Using particulate and total nutrient ratios as deficiency indicators (for "extreme," "moderate," and "no" nutrient deficiency, as defined in the caption for Table 4 based on Guildford and Hecky [2000]), moderate P deficiency was often observed at all study sites, whereas moderate N deficiency was uncommon at all sites except Lakes Edward and George (Table 4). However, consistent detection of SRP (nearly always in excess of 5 μg L^{-1}) implies that P is often available in excess. NH_4^+ was generally present at relatively low concentrations (often below 5 μg L^{-1}, particularly in Lakes Edward, George, and Nkuruba), which, along with the very low nitrate concentrations observed, suggests low availability of dissolved inorganic N in the water columns of these lakes. PartC to Chl *a* ratios (C : Chl *a*) were lowest in Murchison Bay, Napoleon Gulf, and Lake Saka, and highest in Lake Nkuruba and offshore Lake Edward (Table 4), and were related to mean mixed-layer irradiance ($r = 0.73$, $p < 0.001$).

Mean stable C isotopic ratios ($\delta^{13}C$) for phytoplankton from the study lakes (as an indicator of CO_2 availability for photosynthesis, with higher values indicating lower CO_2 availability) ranged from −26.6‰ ± 3.1‰ in mesotrophic Lake Nkuruba to −9.6‰ ± 1.6‰ in hypereutrophic Lake George (Table 2) and were positively related to Chl *a* concentrations ($r = 0.72$, $p < 0.001$). However, phytoplankton from Lake Saka had low $\delta^{13}C$ values relative to the other highly productive study sites (−20.7‰ ± 1.5‰). Chlorophyll-normalized $\delta^{13}C$ values, our index of C limitation, were lowest (less than −24‰ (log Chl $a)^{-1}$) at Edward Open and Lake Nkuruba, between −13‰ and −10‰ (log Chl $a)^{-1}$ in Napoleon Gulf and Lake Saka, and highest (more than −10‰ (log Chl $a)^{-1}$) at Edward Kazinga, Lake Mburo, Murchison Bay, and Lake George.

Microcystin—Over the study period, total MC concentrations ranged from a low of 0.1 μg L^{-1} in Lake Nkuruba to a high of 166 μg L^{-1} in Lake Saka (Table 5; Fig. 2). At all sites, dissolved MC was present at very low concentrations, and on average, cell-bound toxin accounted for

Table 3. Pearson's correlation coefficients (r) for correlations between physical, chemical and biological variables (using all data, $n = 55$). Values with asterisks are significant at the $p < 0.01$ level. Abbreviations are defined in the text or previous table captions, with the exception of: SD^{-1}, inverse Secchi depth; Light, mean mixed-layer light intensity; $\delta^{13}C$ Chl^{-1}, chlorophyll-normalized phytoplankton $\delta^{13}C$; TB, total phytoplankton biomass; CB, Cyanophyta biomass; and MB, *Microcystis* spp. biomass.

	Depth	Temp	SD^{-1}	Light	TP	TN	SRP	NH_4^+	Chl *a*	PartC: PartN	PartC: PartP	PartN: PartP	TN : TP	C : Chl	$\delta^{13}C$ Chl^{-1}	TB	CB	MB	MC
Temp	−0.29	—																	
SD^{-1}	−0.82*	0.03	—																
Light	0.04	−0.16	0.12	—															
TP	−0.79*	−0.02	0.89*	−0.12	—														
TN	−0.27	0.01	0.32	−0.03	0.27	—													
SRP	−0.15	−0.18	0.13	0.07	0.21	0.34	—												
NH_4^+	0.02	−0.08	−0.13	−0.27	0.09	−0.01	−0.43*	—											
Chl *a*	−0.73*	0.07	0.75*	−0.43*	0.82*	0.35*	0.04	0.23	—										
PartC : PartN	−0.25	0.28	0.28	0.62*	0.08	−0.03	0.13	−0.52*	−0.19	—									
PartC : PartP	−0.15	−0.01	0.36*	0.04	0.24	0.14	0.14	−0.26	0.32	0.21	—								
PartN : PartP	0.03	−0.20	0.16	−0.37*	0.17	0.16	0.04	0.10	0.42*	−0.47*	0.76*	—							
TN : TP	0.35*	0.03	−0.37*	0.06	−0.50*	0.70*	0.14	−0.03	−0.29	−0.09	−0.05	0.02	—						
C : Chl	0.16	0.05	−0.04	0.71*	−0.25	−0.19	0.14	−0.46*	−0.59*	0.78*	0.09	−0.43*	0.01	—					
$\delta^{13}C$ Chl^{-1}	−0.65*	0.19	0.64*	−0.52*	0.71*	0.35	−0.07	0.24	0.85*	−0.25	0.07	0.21	−0.31	−0.63*	—				
TB	−0.70*	0.01	0.67*	−0.21	0.73*	0.17	0.17	−0.03	0.74*	−0.02	0.33	0.32	−0.39*	−0.30	0.70*	—			
CB	−0.51*	−0.09	0.60*	−0.29	0.67*	0.16	0.13	0.03	0.74*	−0.21	0.35	0.46*	−0.36*	−0.38*	0.68*	0.93*	—		
MB	−0.49*	−0.21	0.40*	−0.23	0.53*	0.12	0.05	0.20	0.72*	−0.27	0.27	0.45*	−0.23	−0.36	0.65*	0.61*	0.62*	—	
MC	−0.61*	−0.17	0.60*	−0.37*	0.74*	0.32	0.25	0.22	0.80*	−0.26	0.27	0.42*	−0.26	−0.49*	0.67*	0.67*	0.67*	0.70*	—
Cell quota	0.40*	−0.17	−0.37	−0.03	−0.28	−0.11	0.14	−0.07	−0.41*	0.02	−0.18	−0.22	0.08	0.03	−0.52*	−0.37	−0.39*	−0.49*	0.02

> 90% of total MC at all sites except Edward Open (77%), Napoleon Gulf (89%), and Lake Nkuruba (55%).

MC concentrations were related to several environmental variables. Across all lakes there were positive relationships between MC and Chl *a*, TP, PartP, PartSi, particulate N : P ratios, and chlorophyll-normalized $\delta^{13}C$ values (Table 3) and negative relationships between MC and both site depth and SD^{-1}. One of the strongest predictors for MC concentrations was *Microcystis* spp. biomass (Fig. 2d, $r = 0.70$, $p < 0.001$), and seasonal changes in MC concentrations generally coincided with changes in *Microcystis* biomass (A. E. Poste unpubl. data).

Microcystis spp. biomass was positively correlated with Chl *a* (Fig. 2c), TP, PartP, and chlorophyll-normalized $\delta^{13}C$, and negatively correlated with SD^{-1} and sampling site depth (Table 3). Although not a linear relationship, *Microcystis* spp. biomass tended to be lower where mean mixed-layer irradiance was higher; however, below a mean mixed-layer irradiance level of ~ 4 mmol photons m^{-2} min^{-1}, *Microcystis* spp. biomass tended to decline with decreasing irradiance (Fig. 3a). Similarly, the proportion of total phytoplankton biomass present as *Microcystis* peaked at 4 mmol photons m^{-2} min^{-1} (Fig. 3b). MC concentrations were negatively related to both C : Chl *a* ($r = -0.52$, $p < 0.001$) and mean mixed-layer irradiance.

MC cell quotas (expressed as femtograms MC per cell *Microcystis* spp.) ranged from 0.8–517 fg $cell^{-1}$ over the study period (Table 5). Cell quotas were not calculated for Lake Nkuruba as *Microcystis* spp. was not present and MC concentrations were very low in this lake. Cell quota of MC was negatively related to *Microcystis* spp. biomass, Chl *a*, total phytoplankton biomass, Cyanophyta biomass, and phytoplankton $\delta^{13}C$ (Table 3). Using untransformed *Microcystis* biomass data, we found a negative exponential relationship between MC cell quota and *Microcystis* biomass. Positive relationships were observed with site depth and SD^{-1}. Also, for Napoleon Gulf, we observed a positive relationship between cell quota and mean mixed-layer light intensity ($r = 0.74$, $p < 0.05$, df = 8); however, this relationship was not seen at any other study site. We also observed a negative relationship between cell quota and chlorophyll-normalized phytoplankton $\delta^{13}C$ (Table 3; Fig. 3d), our indicator of C availability relative to demand, suggesting that diurnal C limitation may moderate cellular MC content.

Discussion

Physicochemical conditions and limitation of phytoplankton biomass—The current study primarily included shallow, nearshore regions of large lakes and smaller shallow lakes, where whole water column mixing prevailed throughout the study period (with the exception of Lake Nkuruba, where depth [38 m], small surface area, and a protected location within a steep-walled crater are all barriers to complete mixing). These conditions allow for relatively stable phytoplankton community biomass and taxonomic composition compared to temperate lakes, where there is strong seasonality in temperature, solar irradiance, and mixing.

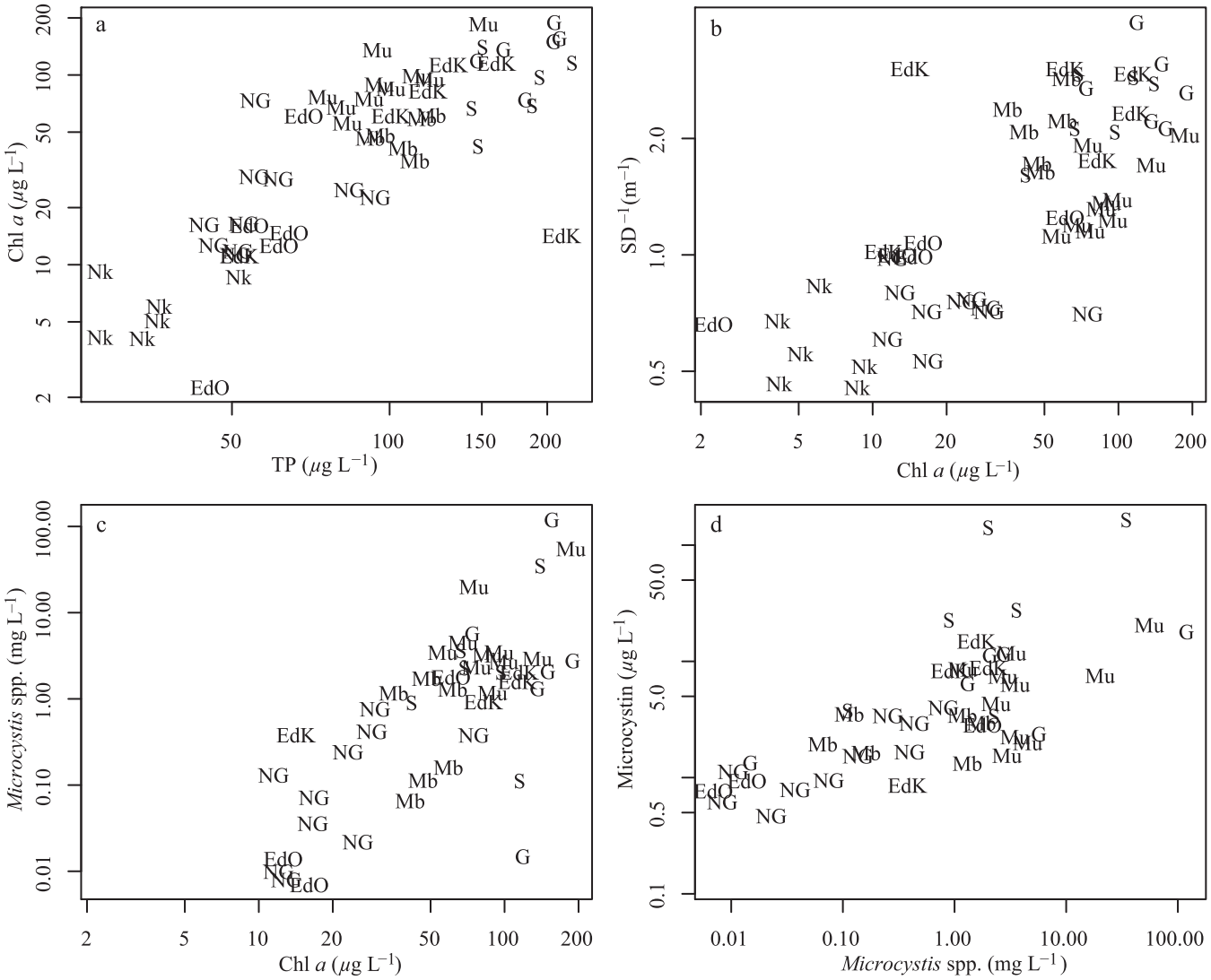

Fig. 2. Scatterplots for: (a) Chl *a* and TP (r = 0.82), (b) inverse Secchi depth (SD^{-1}) and Chl *a* (r = 0.75), (c) Chl *a* and *Microcystis* spp. biomass (r = 0.72), and (d) *Microcystis* spp. biomass and MC concentrations in water (r = 0.70). Site codes are given in Table 1.

Using the trophic status classification system outlined in Vollenweider and Kerekes (1982), based on SD, TP, and Chl *a*, the shallowest sites (George, Saka, Mburo, Edward Kazinga, and Murchison Bay) were hypereutrophic, and the deepest sites were eutrophic (Edward Open and Napoleon Gulf) and mesotrophic (Nkuruba). This is likely because the shallowest sites experience regular whole water column mixing with attendant rapid nutrient recycling from the sediments into a shallow water column with limited dilution capacity. Also, these shallow sites allow for a higher standing crop of phytoplankton to develop before becoming limited by self-shading (Silsbe et al. 2006). Based on particulate and total nutrient ratios, there was no evidence for strong N or P limitation at most sites (Table 4); this, combined with the consistently detectable dissolved nutrient concentrations (particularly SRP) at all sites, implies that nutrient concentrations are not the primary limiting factor for phytoplankton biomass in these systems.

Although NH_4^+ and NO_3^- concentrations were generally low at our study sites, given that moderate P deficiency was often observed, while moderate N deficiency was uncommon at all sites except Lakes Edward and George (Table 4), it is likely that N-fixation (or acquisition of benthic N sources by buoyancy-regulating cyanobacteria) may be meeting any deficiency in dissolved inorganic N at most sites. This suggests that P is more likely than N to eventually limit phytoplankton maximum biomass (Schindler 1977; Hecky et al. 2010), even in these P-rich systems. N-fixation is thought to account for up to 80% of external N inputs to Lake Victoria (Mugidde et al. 2003) and more than half of the total N input to Lake George (Horne and Viner 1971), and this process is likely to play an important role in N dynamics at all of our sites.

The light environment at these study sites was largely determined by phytoplankton biomass (Fig. 2b), and mean mixed-layer irradiance was often at or near the range where

Table 4. Particulate carbon, nitrogen, and phosphorus concentrations, particulate nutrient ratios, and carbon to chlorophyll *a* ratios for the study lakes (using all data, $n = 55$). Sites are arranged by depth (as in Table 1), and results are reported in the format of mean ± standard deviation. Particulate carbon : chlorophyll *a* (C : Chl) in units of μmol C μg Chl a^{-1}.

Site	PartC (μg L^{-1})	PartN (μg L^{-1})	PartP (μg L^{-1})	PartC : PartN (molar)	PartC : PartP (molar)	PartN : PartP (molar)	TN : TP (molar)	C : Chl
George	24,854±6113	3080±429	287.3±90.8	9.3±1.2	232.2±54.3	24.8±4.5	16.1±10.3	16.3±7.0
Saka	11,387±3635	1817±746	161.0±45.3	7.5±0.8	186.0±50.1	25.3±9.4	32.3±22.4	11.1±1.7
Mburo	9796±2423	1391±266	149.9±34.0	8.2±0.7	172.5±42.5	21.1±5.0	42.0±25.1	17.0±3.4
Edward Kazinga	12,042±7084	1509±942	201.6±116.0	9.6±1.0	152.5±34.9	16.2±4.5	33.2±15.8	18.7±7.0
Murchison Bay	6812±2971	1229±492	104.0±33.0	6.5±0.2	175.2±51.4	27.3±7.9	50.2±22.7	6.0±1.3
Edward Open	8783±10,533	1006±1287	128.1±149.0	11.0±1.1	172.4±15.5	15.9±2.4	37.7±6.6	40.8±25.0
Napoleon Gulf	2608±646	476±155	50.7±8.2	6.5±0.6	136.2±24.3	21.2±5.1	62.2±41.3	9.3±2.8
Nkuruba	1637±454	773±1307	24.0±3.4	7.7±0.8	179.5±55.7	23.1±5.9	81.6±39.7	24.5±11.2

Potential nutrient deficiency was assessed from particulate nutrient ratios based on the criteria outlined by Guildford and Hecky (2000; based on Healy and Hendzel 1980) and summarized as follows: (1) based on PartC : PartN: < 8.3 (no N deficiency), 8.3–14.6 (moderate N deficiency), and > 14.6 (extreme N deficiency); (2) based on PartC : PartP: < 129 (no P deficiency), 129–258 (moderate P deficiency), > 258 (extreme P deficiency); (3) based on PartN : PartP: < 22 (no P deficiency), > 22 (P deficiency); (4) based on TN : TP: < 20 (N deficiency), 20–50 (either N or P deficiency), and > 50 (P deficiency).

light limitation of phytoplankton growth could occur. In Napoleon Gulf and Murchison Bay, consistently low mean irradiance values suggest that light limitation may be common, with thermal stratification offering occasional relief from low-light conditions in the mixed layer of Napoleon Gulf. Despite having comparable and often much higher phytoplankton biomass than Murchison Bay or Napoleon Gulf, in Lakes George, Mburo, and Saka, shallow depths allow mean water column irradiances to exceed those observed for the Lake Victoria sites. These low-light conditions, paired with the observation that nutrient availability does not appear to strongly limit phytoplankton biomass in these lakes, suggest that light limitation through self-shading is likely to be an important determinant of phytoplankton biomass in these lakes.

Factors affecting cyanobacterial dominance and abundance—The contribution of cyanobacteria to the total phytoplankton biomass tended to be highest at the hypereutrophic stations and lower at the mesotrophic and eutrophic stations (Edward Open and Napoleon Gulf), with the exception of Lake Nkuruba where cyanobacteria (primarily *Planktolyngbya* spp. and *Cylindrospermopsis* spp.) made up nearly all of the total biomass. Several cyanobacterial taxa known to be capable of MC production were prevalent in the study lakes, including *Microcystis* spp., *Planktothrix* spp., *Anabaena* spp., and *Cylindrospermopsis* spp.

Many hypotheses have been put forward in the literature with respect to the physicochemical characteristics that favor high cyanobacterial abundance in freshwater lakes (Paerl 1988; Zurawell et al. 2004). However, while many of these hypotheses have been tested for temperate lakes, few studies have sought to determine whether the same processes and drivers apply in tropical settings. We have tested several of these hypothetical drivers of cyanobacterial abundance against our field observations from Ugandan lakes; specifically, we have examined whether cyanobacterial abundance is favored by: high water column stability, temperature, pH, N or P concentrations or low N : P ratios, SRSi, CO_2 availability, or light availability.

High water column stability and high temperature?: Water column stability is thought to favor cyanobacterial genera that are capable of buoyancy regulation (Paerl and Huisman 2009). Given that the highest cyanobacterial (and *Microcystis*) biomasses were observed for shallow, well-mixed study sites, water column stability was not an important determinant of cyanobacterial abundance in these lakes. However, diurnal stratification of these waters may provide the water column stability necessary for cyanobacteria capable of buoyancy regulation to outcompete other taxa for light and nutrients, and *Microcystis* blooms are commonly observed in shallow, well-mixed temperate and subtropical lakes (Dokulil and Padisák 1994; Chen et al. 2003). Many cyanobacterial taxa are also known to thrive at high water temperatures relative to other types of phytoplankton (Paerl and Huisman 2009), and the high temperatures encountered in these tropical lakes may play a role in the cyanobacterial dominance of these systems.

Table 5. Summary of mean (± standard deviation) *Microcystis* biomass, *Microcystis* cell numbers, % of cyanobacterial biomass as *Microcystis*, % of total biomass as *Microcystis*, MC concentrations (in whole water), and MC cell quota. Data ($n = 49$) are from all sites except for Lake Nkuruba, where no *Microcystis* was observed and concentrations of MC were consistently low (0.12–0.26 μg L^{-1}). Sites are arranged by depth (as in Table 1).

Site	*Microcystis* biomass (mg L^{-1})	*Microcystis* ($\times 10^9$ cells L^{-1})	% of cyanobacterial biomass	% of total biomass	MC (μg L^{-1})	Cell quota (fg MC cell *Microcystis*$^{-1}$)
George	22.1±48.3	10.15±17.08	31.3±30.8	29.7±30.3	8.54±6.36	7.8±9.9
Saka	7.4±13.7	1.32±2.04	34.3±29.4	22.0±23.2	61.2±73.4	112.6±171.1
Mburo	0.7±0.6	8.61±6.91	11.6±8.6	6.1±5.1	2.48±0.96	19.0±34.3
Edward Kazinga	0.82±0.85	0.30±0.39	19.5±19.0	12.2±15.1	5.81±5.86	24.9±20.0
Murchison Bay	9.9±16.9	4.82±7.78	67.4±13.7	47.6±18.7	7.26±5.73	9.8±11.9
Edward Open	0.36±0.8	0.11±0.24	4.6±9.5	4.0±8.9	0.97±1.10	165.1±149.7
Napoleon Gulf	0.2±0.3	0.06±0.08	8.1±7.5	5.9±6.0	1.75±1.26	119.5±164.3

High nutrient concentrations? Low TN : TP ratios?: Phosphorus and nitrogen concentrations have been identified as important determinants of cyanobacterial dominance and abundance (Trimbee and Prepas 1987; Paerl 1988; Downing et al. 2001). The strong positive relationships observed between both cyanobacterial and *Microcystis* biomass and TP, and the lack of relationship between these variables and TN suggests that P, but not N, concentrations are of particular importance in determining cyanobacterial abundance, even of non–N-fixing cyanobacteria such as *Microcystis*. The abundance of cyanobacteria in freshwater systems is often negatively related to TN : TP ratios (Smith 1983), which has been attributed to the fact that many cyanobacterial taxa are capable of atmospheric N fixation, while those that cannot fix atmospheric N (such as *Microcystis*) are often effective competitors for N, and can use buoyancy regulation to access benthic N sources (Paerl 1988). These N acquisition strategies may be of particular relevance to our study systems, where dissolved inorganic N concentrations in the water column were typically low. Among these tropical lakes, the highest cyanobacterial biomass tended to be coincident with the lowest TN : TP ratios, supporting the hypothesis that low N availability relative to P may favor cyanobacteria.

Low SRSi concentrations?: Eutrophication-mediated silica depletion and subsequent limitation may have played a role in the shift from dominance of phytoplankton communities by large diatoms to cyanobacteria in the Laurentian Great Lakes (Schelske and Stoermer 1971) as well as Lake Victoria (Kling et al. 2001; Hecky et al. 2010). Despite relatively high SRSi concentrations in the study lakes (with the exception of the Lake Victoria sites), only in well-mixed and relatively transparent Lake Edward did diatoms consistently make up a substantial proportion of the total phytoplankton biomass. At the remaining study lakes, diatom abundance is likely limited by the prevalence of buoyancy-regulating cyanobacteria that can monopolize light and outcompete more rapidly settling diatoms (in Lakes Saka, George, Mburo, and Murchison Bay), or lack of turbulent mixing (in Lake Nkuruba). As such, SRSi limitation is unlikely to be a primary driver of cyanobacterial dominance in our study lakes, with the possible exception of Napoleon Gulf and Murchison Bay.

Low light conditions?: Many cyanobacterial taxa are capable of maintaining a higher growth rate than other groups of phytoplankton in low light conditions (Mur 1983). *Planktolyngbya*, known to be tolerant of low light (Reynolds 2006), dominated the cyanobacterial biomass in most of the study lakes, while despite relatively high light requirements, *Microcystis* was also prevalent in these low-light systems, likely due to its ability to regulate buoyancy and rise to the upper levels of the water column where light is not limiting. Mean mixed-layer light intensity did not show a linear relationship with cyanobacterial or *Microcystis* biomass; however, there was a negative relationship between *Microcystis* (and cyanobacterial) biomass and mean mixed-layer light intensity above 4 mmol photons m^{-2} min^{-1}, likely due to the association between high transparency and low biomass of *Microcystis* (typically under lower nutrient conditions) as well as the ability of *Microcystis* to thrive under low light conditions where total phytoplankton biomass is high. However, below ~ 4 mmol photons m^{-2} min^{-1}, *Microcystis* biomass (and relative biomass of *Microcystis*) also declined, suggesting limitation of this cyanobacterial taxon by light availability below this threshold (Fig. 3a,b). In these tropical study lakes, high biomasses of cyanobacteria, sustained by high nutrient concentrations, lead to low light conditions, which in turn may favor cyanobacterial competitive dominance (but not necessarily *Microcystis*) as light becomes very low.

Low CO_2, high pH?: Cyanobacteria are also known to be effective competitors for dissolved inorganic C (Shapiro 1973; Paerl 1988; Shapiro 1997), with several taxa (including *Anabaena* and *Microcystis*) capable of bicarbonate uptake when CO_2 availability is limited (Paerl 1988). There is also evidence that buoyancy-regulating cyanobacteria can access CO_2 by rising to surface waters (Paerl 1988). Based on Okello and Kurmayer (2011), pH in our study lakes is often above 8 or even 9, suggesting that CO_2 availability is quite low. Furthermore, phytoplankton from many of these lakes (particularly where cyanobacterial biomasses were high) were strongly ^{13}C enriched, which indicates reduced isotopic discrimination during photosynthesis under CO_2-limited conditions as well as possible cyanobacterial use of bicarbonate as a C source. This evidence from tropical lakes supports the hypothesis (based on results from temperate

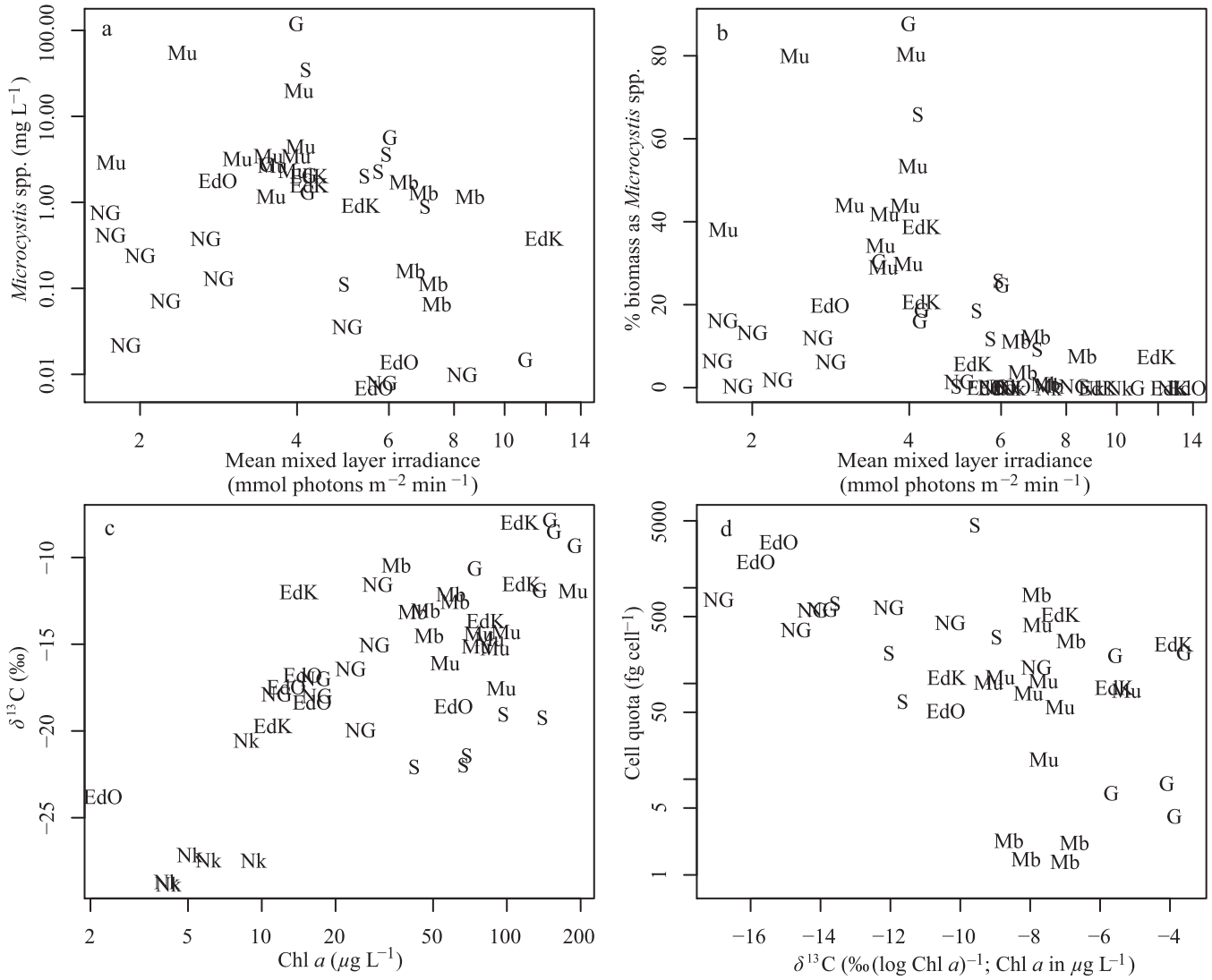

Fig. 3. Scatterplots for: (a) *Microcystis* spp. biomass and mean mixed-layer light intensity, (b) proportion of total phytoplankton biomass that is present as *Microcystis* spp., (c) phytoplankton δ^{13}C and Chl *a* concentrations ($r = 0.72$), and (d) cell quota of MC and chlorophyll-normalized phytoplankton δ^{13}C values ($r = -0.52$). Site codes are given in Table 1.

lakes) that low-CO_2 and high-pH conditions, particularly in highly productive lakes, may favor cyanobacterial dominance.

MC dynamics—The strongest predictor for MC concentrations in these lakes was lake trophic status, with higher-trophic-status lakes (where chlorophyll and nutrient concentrations were high and transparency was low) having the highest *Microcystis* biomass and subsequent MC concentrations (Fig. 2). While MC concentrations were positively related to *Microcystis* biomass, they were not related to the biomass of any other cyanobacterial taxa known to be capable of MC production (e.g., *Planktothrix*, *Anabaena*, *Cylindrospermopsis*), suggesting that *Microcystis* spp. was the dominant MC producer across all study sites. This differs from temperate lakes, where in addition to *Microcytsis*, *Planktothrix* (including *Planktothrix agardhii* and *Planktothrix rubescens*) is often an important MC-producing taxon (Kurmayer and Gumpenberger 2006); however, as in our Ugandan study lakes, *Anabaena* is rarely a substantial contributor to MC production in temperate lakes.

MC cell quotas observed at all study sites fell within the range of values reported in the global literature, and were similar to those previously reported for similar study sites by Okello et al. (2010), with the highest cell quotas observed in Napoleon Gulf and Lake Saka, and the lowest cell quotas observed in Lake George (Table 5). We tested several hypotheses derived from the temperate freshwater literature regarding the factors that influence MC cell quota.

Presence and prevalence of cyanobacteria capable of microcystin production: Our observation that *Microcystis*

was the primary MC-producing taxon is consistent with previous observations of regular occurrence of the *mcy*B genotype responsible for MC production in *Microcystis* populations from several of our study lakes (Okello et al. 2010). In Lake Nkuruba, MC was detectable (albeit at very low levels) despite the absence of *Microcystis* spp. in the lake, suggesting that some other cyanobacterial genera may have been responsible for MC production. The strongest predictors for *Microcystis* spp. biomass overlapped a great deal with the predictors for MC concentrations, suggesting that rather than controlling MC concentrations directly, these variables may be indirectly influencing MC concentrations through effects on *Microcystis* spp. biomass (Fig. 2). This is consistent with observations from temperate systems, where biomass of toxin-producing cyanobacteria is often the primary determinant of toxin concentrations (Giani et al. 2005; Rinta-Kanto et al. 2009).

For all of the study lakes included in the current study (with the exception of Lake Nkuruba), Okello et al. (2010) observed a strong positive relationship between MC cell quota and the proportion of the *mcy*B genotype in the *Microcystis* population. As such, it is likely that much of the difference in cell quota between sites may be attributable to differences in the genotypic composition of *Microcystis* (and the resulting capacity for MC production).

Environmental effects on microcystin production by toxin-producing strains: Factors likely to regulate *Microcystis* growth, such as the availability of light and nutrients, may also limit MC production, given the evidence that higher cell quotas tend to be observed at higher cell growth rates (Orr and Jones 1998; Sivonen and Jones 1999; Deblois and Juneau 2010). The negative exponential relationship that we observed between MC concentrations and *Microcystis* cell quota (based on untransformed data) could suggest that at high *Microcystis* biomass, cellular MC production may decrease, indicating that something may become limiting for MC production as *Microcystis* approaches maximum biomass and cell growth rates decline. However, this relationship could also reflect higher cellular MC loss rates at high *Microcystis* biomass.

Nutrients: Given the lack of evidence for strong N or P deficiency of phytoplankton in this study as well as the lack of relationships between nutrient concentrations and ratios and cell quota of MC, limitation by these nutrients is unlikely to play an important role in the determination of MC concentrations in these lakes.

Light: Several studies have reported negative relationships between photon irradiance and MC cell quota (Wiedner et al. 2003; Deblois and Juneau 2010). However, this relationship was observed at high photon irradiances, where cellular production of MC as well as photosynthesis may be inhibited, and these studies observed an increase in cell quota of MC with increasing light up to the point where maximum growth rate was achieved (Wiedner et al. 2003; Deblois and Juneau 2010). In Napoleon Gulf, the positive relationship between cell quota of MC and mean mixed-layer light intensity suggests that in this low-light environment, where mean mixed-layer light intensity was consistently lower than at any other site (below 3 mmol photons m^{-2} min^{-1} on seven of the 10 sampling occasions), light limitation of cell growth rate may affect MC production and subsequent cell quotas.

CO_2: Several studies have shown that in productive systems, photosynthesis as well as phytoplankton growth rates can be diurnally limited by the availability of inorganic C (Schindler and Fee 1973; Shapiro 1973), and diurnal drawdown of dissolved inorganic C has been observed in nearshore Lake Victoria (Ramlal et al. 2001). However, despite the potential for instantaneous inorganic C limitation of both photosynthesis and phytoplankton growth rates, there is no evidence that inorganic C availability moderates the total phytoplankton biomass in freshwater systems (Schindler 1977). In the current study, we use stable C isotopic ratios ($\delta^{13}C$, as an indicator of CO_2 availability) normalized to Chl *a* (as an indicator of photosynthetic demand for CO_2) to describe CO_2 availability relative to demand to test whether C availability can affect cellular MC concentrations. The decrease in MC cell quota with increasing chlorophyll-normalized $\delta^{13}C$ values (Fig. 3d) suggests that MC cell content is highest where the likelihood of C limitation of photosynthesis is lowest, and tends to decrease as C limitation becomes more likely. Of particular interest is Lake Saka, where despite hypereutrophic conditions, phytoplankton $\delta^{13}C$ as well as pH (Okello and Kurmayer 2011) tended to be relatively low, suggesting that CO_2 may be more available in Lake Saka compared to other hypereutrophic lakes, possibly as a result of high rates of decomposition of organic matter in this lake (which receives a great deal of agricultural and human waste from its catchment). Chlorophyll-normalized phytoplankton $\delta^{13}C$ values for Lake Saka were similar to those observed for Napoleon Gulf, perhaps explaining why the MC cell quotas for these two sites were very similar despite quite different environmental conditions. In Lake Saka, high *Microcystis* biomass may combine with the elevated cell quota possible under reduced CO_2 deficiency to result in total MC concentrations that occasionally exceed 100 μg L^{-1} (and are, on average, nearly seven times higher than at any other site). In a study of laboratory cultures, Jähnichen et al. (2007) concluded that MC might play a role in the C-concentrating mechanism of cyanobacteria, thereby allowing *Microcystis* in culture to overcome C limitation of growth. Our results in extremely hypereutrophic lakes may indicate that, if MC plays this role at the cellular level, there may be limits to the ability of MC synthesis to sustain growth rates when C is strongly limiting.

By affecting cell growth rates, C availability may influence the amount of MC being produced by cyanobacteria. MC synthesis may be initially stimulated by the onset of C limitation in these eutrophic lakes; however, as phytoplankton growth rates fall cellular MC production may also decline as cells shift energy synthesis increasingly into cell maintenance rather than net growth. This suggests that, although inorganic C availability is not expected to influence phytoplankton biomass in these Ugandan lakes as CO_2 invasion at night can give a daily replenishment to sustain

biomass growth (Schindler 1977), the potential for diurnal C limitation of photosynthesis and phytoplankton growth rates is not only of theoretical interest but of practical relevance, as it may play a role in determining cyanobacterial toxicity. These novel results highlight the need for future field-based research to test the utility of chlorophyll-normalized $\delta^{13}C$ as an indicator of C limitation and to improve our understanding of the interactions between C availability, competitive success of *Microcystis*, and MC dynamics.

General explanatory framework for predicting MC concentrations: Based on the results of this study, we propose a general explanatory framework for occurrence of MC at these Ugandan study sites whereby: (1) at shallow sites, continuous recycling of nutrients from the sediments into well-mixed water columns with limited dilution capacity leads to high P concentrations; (2) high P concentrations lead to high phytoplankton (and particularly cyanobacterial) biomass; (3) phytoplankton growth (and biomass) is eventually light limited due to self-shading and occasionally P limited at maximum biomass, with shallower sites able to support higher phytoplankton biomass under self-shading conditions; (4) low light (and possibly low CO_2) conditions favor *Microcystis* spp., which is able to regulate its position in the water column through buoyancy control and access resources that limit other taxa; (5) MC concentrations are determined by *Microcystis* biomass but also by the cell quota of MC; and (6) MC cell quota is determined by the genotypic composition of *Microcystis* as well as *Microcystis* growth rate (which may be moderated by diurnal C limitation or light availability). This chain of causation explains the elevated MC concentrations (and *Microcystis* biomass) at the shallowest study sites (Edward Kazinga, Lake George, Lake Mburo, and Murchison Bay) and the remarkably high concentrations in Lake Saka.

The drivers identified in the current study for cyanobacterial abundance and MC concentrations in tropical East African lakes are very similar to those that have been proposed for temperate lakes based on extensive field and laboratory-based studies. This suggests that much of our current understanding of the environmental conditions and processes that encourage MC-producing blooms of cyanobacteria (especially *Microcystis* spp.) in temperate systems applies to tropical lakes as well. In particular, the importance of P concentrations in driving cyanobacterial abundance (and subsequent MC concentrations) should focus efforts to reduce MC risk on reducing P concentrations in tropical as well as temperate lakes.

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