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Community Biological Ammonium Demand: A Conceptual Model for Cyanobacteria Blooms in Eutrophic Lakes

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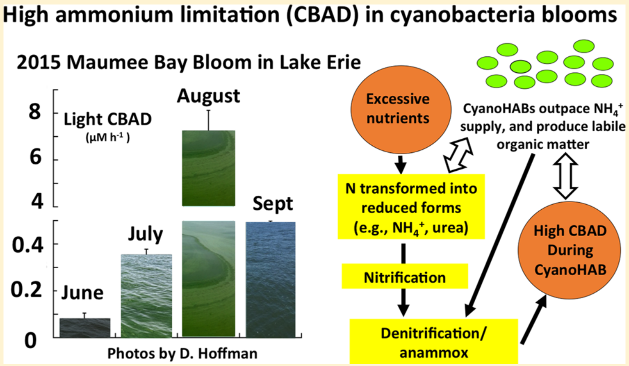
Supporting Information

ABSTRACT: Cyanobacterial harmful algal blooms (CyanoHABs) are enhanced by anthropogenic pressures, including excessive nutrient (nitrogen, N, and phosphorus, P) inputs and a warming climate. Severe eutrophication in aquatic systems is often manifested as non-N_2-fixing CyanoHABs (e.g., *Microcystis* spp.), but the biogeochemical relationship between N inputs/dynamics and CyanoHABs needs definition. Community biological ammonium (NH_4^+) demand (CBAD) relates N dynamics to total microbial productivity and NH_4^+ deprivation in aquatic systems. A mechanistic conceptual model was constructed by combining nutrient cycling and CBAD observations from a spectrum of lakes to assess N cycling interactions with CyanoHABs. Model predictions were supported with CBAD data from a *Microcystis* bloom in Maumee Bay, Lake Erie, during summer 2015. Nitrogen compounds are transformed to reduced, more bioavailable forms (e.g., NH_4^+ and urea) favored by CyanoHABs. During blooms, algal biomass increases faster than internal NH_4^+ regeneration rates, causing high CBAD values. High turnover rates from cell death and remineralization of labile organic matter consume oxygen and enhance denitrification. These processes drive eutrophic systems to NH_4^+ limitation or colimitation under warm, shallow conditions and support the need for dual nutrient (N and P) control.



INTRODUCTION

Harmful algal blooms (HABs) have increased in nutrient polluted lakes, estuaries, and coastal ecosystems worldwide, often resulting in disruptions of ecosystem services.[1−4] Nitrogen (N) and phosphorus (P) are critical to the development, composition, and persistence of HABs, but their roles in modulating HABs differ substantially (Supporting Information, SI). Exact mechanisms of N transformations relative to bloom dynamics remain unclear, but ammonium (NH_4^+) availability helps drive phytoplankton community structure[5,6] and toxicity,[7] whereas low NH_4^+ concentrations prevent toxin production.[8]

Currently, nutrient limitation is estimated from nutrient ratios of suspended material (i.e., elemental stoichiometry of seston[9]) or nutrient addition and dilution bioassays (e.g.,[10−12] SI). We complement these useful efforts by examining NH_4^+ dynamics and the degree of community biological NH_4^+ demand (CBAD) over diel cycles under natural light and dark conditions. Isotope dilution experiments[13−15] estimate NH_4^+ cycling rates (potential uptake and actual regeneration

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Table 1. Site Characteristics and Sampling Dates

site name	abbreviation	sampling dates	avg Chl (μg/L)	chl method	T (°C)	data source
Lake Michigan	LMI	Jun 1999−May 2000	2.44	extracted	0.6−17.5	Gardner et al. 2004[29]
Lake Rotorua	RUA	Jan 2006	23.3	sonde	20.7	McCarthy et al., unpublished
Lake Rotoiti	ITI	Jan 2006	7.66	sonde	21.7	McCarthy et al., unpublished
Missisquoi Bay	MBLC	Jun-Sep 2007−2009	16.2	extracted	11.4−23.9	McCarthy et al. 2013[30]
W. Lake Erie	WLE	Jul 2004, Sep 2005	4.23	extracted	19.3−20.9	Moats 2006[31]
Lake Okeechobee	LO	May 2009	16.8	extracted	26.3	DBHYDRO, James et al. 2011[32]
Taihu Lake	TL	Sep and Oct 2002, May 2004	11.5	extracted	5.8−23.8	McCarthy et al. 2007,[21] Paerl et al. 2011[33]
Old Woman Creek	OWC	Jul 2003, Jul 2004	14.0	extracted	20.2−23.4	McCarthy et al. 2007[34]
Lake Maracaibo	MBO	Sep 1995	7.76	extracted	30.2−31.2	Gardner et al. 1998[26]
W. Lake Erie Bloom	WLE Bloom	Aug 2015	209	extracted	23.9−24.1	Hoffman et al., unpublished

rates). CBAD characterizes internal NH_4^+ cycling by representing the difference between measured potential NH_4^+ uptake rates and actual NH_4^+ recycling rates (NH_4^+ regeneration rates) in aquatic systems.

Labile organic matter, used by heterotrophic organisms (and cyanobacteria in some cases) to support growth and energy demands, consists of reactive organic substances (e.g., amino acids, urea, carbohydrates, proteins, and other organic metabolites), which are transformed rapidly via grazing, autolysis of organic particles, and assimilation of dissolved organic materials by microorganisms[16] or oxidized by cell surface oxidases.[17] Degradation of labile organic compounds affects ecosystem carbon, oxygen, and nitrogen dynamics by removing O_2 and releasing NH_4^+ and other bioavailable nutrients. Significant amounts of N-rich organic compounds are produced and processed in eutrophic ecosystems, but their dynamics are often underestimated because they are assimilated and metabolized rapidly (e.g., refs 18 and 19). Ammonium cycling reflects microbial processes, including labile organic N degradation rates, from metabolic excretion or mineralization. Ammonium is also an important N substrate for primary productivity, including HAB growth, coupled nitrification/denitrification, and anaerobic ammonium oxidation (anammox[20−23]). Understanding the functions of NH_4^+ and labile organic matter is crucial to determining the extent to which reduced N compounds impact the initiation, maintenance, and toxicity of blooms.

The degree and type of N loading and the formation/remineralization of labile organic N create conditions conducive for cyanobacterial HABs (CyanoHABs), which thrive at warm temperatures.[11,24] Here, we address the following questions: 1. Does CBAD reflect NH_4^+ limitation development during CyanoHABs? 2. Do CyanoHABs have an unmet NH_4^+ demand (CBAD) in eutrophic lakes? 3. What are the biogeochemical mechanisms relating CBAD to CyanoHABs? We examined CBAD in a variety of lakes, including hypereutrophic, large lakes Maracaibo (Venezuela), Taihu (China), and Erie (USA). The resulting conceptual model, developed from these previous studies, was tested in Maumee Bay, Lake Erie, before, during, and after a cyanobacterial (*Microcystis*) bloom in the summer of 2015. Results illustrate how excessive external nutrient inputs lead to regional CyanoHABs and corresponding high CBAD at warm temperatures in shallow, eutrophic aquatic ecosystems.

■ STUDY SITES

Average CBAD values were calculated and compared from potential uptake and actual regeneration (assumed to equal actual recycling rate) data from lakes sampled over a range of times, locations, and latitudes (e.g., ref 20). Photic CBAD (the difference between CBAD in samples incubated at in situ light and dark conditions), indicating NH_4^+ demand from light-dependent organisms, is considered for systems where data were collected over diel incubations but not for Lake Maracaibo or Maumee Bay during an intense CyanoHAB in 2015 due to short (<4 h) incubations. Sampling dates, geochemical parameters, and data sources are reported in Table 1.

Lake Maracaibo is a large (13280 km^2), coastal lake in Venezuela located at the northern tip of South America, about 10° N latitude. In contrast to the lake (salinity of ca. 1.3), the Strait of Maracaibo, connected to the Atlantic Ocean via the Gulf of Venezuela, has regionally variable salinities (1.5−33).[25] The lake is warm (∼30 °C), relatively shallow (mean depth ∼25 m),[25] and hypereutrophic.[26] The northern channel connected to the lake via the Strait of Maracaibo was impacted by direct inputs of untreated sewage (a relatively continuous supply of nutrients) and industrial wastes from the city of Maracaibo (population ca. 1 million).[26] Algal productivity ranged from ca. 3 to 8 g C m^2 d^{-1} in northeastern regions of the lake.[27] Southeastern and southern regions of Lake Maracaibo (e.g., sites 4 and 6, Figure 4) receive about 70% of the watershed runoff through the freshwater Catatumbo River, which drains the surrounding regions (mostly mountain and agricultural;[28]). Sampling sites were selected along a gradient toward the Gulf of Venezuela, with lowest station numbers farthest from the river.

Lakes Taihu (China) and Erie (USA) are large, eutrophic lakes in temperate climates. Lake Taihu is located in the Changjiang (Yangtze) River delta in eastern China. It is ca. 2400 km^2 in area and has a mean depth of ∼2 m. The urban, industrial, and agricultural Taihu Basin accounts for about 11% of China's gross domestic product.[35] Lake Erie has the smallest volume (480 km^3) and is the shallowest (mean depth 19 m) of the Laurentian Great Lakes. It is divided into three distinct basins, each with different geomorphological and biogeochemical characteristics.[36] The western basin is shallow (mean depth 7.6 m) and susceptible to CyanoHABs, especially in Maumee Bay, with high nutrient inputs from the Maumee River (e.g.,[37]). Both lakes are characterized by point and nonpoint sources of nutrients entering shallow, tributary-polluted bays and experience non-N_2 fixing *Microcystis* spp. blooms (Taihu since 1959, Erie since 1995). These blooms can produce cyanotoxins (microcystins), which have contaminated local drinking water supplies.[38,39] Vertical stratification is uncommon in Lake Taihu due to persistent wind mixing. Stratification is brief and episodic in the western basin of Lake Erie. However, the central basin of Lake Erie experiences summer stratification, hypoxia, and occasional CyanoHABs.[37,40]

NITROGEN DYNAMICS CALCULATIONS AND CBAD

The genetic basis of N assimilation patterns in cyanobacteria provides empirical evidence for cyanobacterial competitive abilities for reduced N forms (e.g., 41). These taxa thrive when abundant nutrients become available under high temperatures. The dynamics and fate of incoming and regenerated NH_4^+ provide important information needed to understand occurrences and development of regional CyanoHABs in polluted lakes, because NH_4^+ provides a preferred N substrate for non N_2-fixing cyanobacteria, e.g., *Microcystis*.[42−45] Incorporation of tracer level additions of $^{15}NH_4^+$ substrate into organisms followed by mass spectrometric analysis of particles for ^{15}N[46] is often used to measure NH_4^+ uptake dynamics.[47] In contrast, isotope dilution approaches, used here to measure regeneration and potential uptake rates, involve adding excess $^{15}NH_4^+$ as the reaction product rather than as a potentially limiting substrate (e.g., refs 13−15, 18, 21, and 23). Ammonium isotopes were measured with ammonium isotope retention time shift-high performance liquid chromatography (AIRTS-HPLC)[48] or, more recently, NH_4^+ oxidation membrane inlet mass spectrometry (OX/MIMS).[49] Overall, specific advantages of isotope dilution for studying NH_4^+ dynamics, in addition to nontracer additions of $^{15}NH_4^+$, are small sample volume requirements, a simple field protocol, and potential assessment of CBAD (e.g., ref 23). CBAD measurements do not require isotope dilution experiments, as they depend only on measuring changes in total NH_4^+ concentrations over a specified time interval, but uptake and regeneration measurements support results interpretations relative to microbial community (including phytoplankton) dynamics.

Incubations of $^{15}NH_4^+$-enriched water samples, with measurements of NH_4^+ concentration and isotope ratios under natural light and dark conditions, were used in our studies to determine potential NH_4^+ uptake and actual NH_4^+ recycling rates (measured here as regeneration rates;[13,14]). They are calculated from $^{15}NH_4^+/NH_4^+$ isotope ratio changes (assuming that only $^{14}NH_4^+$ is released through regeneration/remineralization during the relatively short incubations) and total NH_4^+ concentration changes (i.e., production vs consumption).[13,14,23] The approach of determining potential uptake and actual regeneration rates of NH_4^+ from $^{15}NH_4^+$-enriched isotope dilution experiments was described independently by Blackburn[13] and Caperon et al.[14] with slight modifications for our experiments.[23]

CBAD (community biological NH_4^+ demand; $\mu M\ h^{-1}$) is equal to the change in total NH_4^+ concentration over time in water samples spiked with excess NH_4^+

$$\text{CBAD} = ([NH_4^+]_0 - [NH_4^+]_t)/t$$

where $[NH_4^+]_0$ and $[NH_4^+]_t$ are NH_4^+ concentrations (μM) at the beginning and end of incubations, and t is time (h). In addition, CBAD = potential NH_4^+ uptake − actual NH_4^+ regeneration. Photic CBAD, indicating the degree of NH_4^+ limitation driven by phototrophs, is determined as

$$\text{photic CBAD} = \text{light CBAD} - \text{dark CBAD}$$
$$(\text{absolute difference; } \mu M h^{-1})$$

Field isotope dilution experiments were conducted following the work of Gardner et al.[26] by spiking lake water samples in transparent containers (e.g., 70−500 mL polycarbonate or polystyrene bottles) with excess $^{15}NH_4^+$ (relative to measured or expected NH_4^+ concentrations). Incubation bottles were mixed and incubated under natural light and dark (foil- or tape-wrapped) and nearly in situ temperature. Incubation bottles were submerged in lake water on site in Taihu and Erie (ref 21; see the SI for more details) or in ship-board, flow-through incubators for Maracaibo.

Potential NH_4^+ uptake rates represent the capability of plankton to assimilate NH_4^+ when NH_4^+ is available and are assumed here to reflect relative microbial activity for our rate comparisons. Actual NH_4^+ regeneration rates estimate labile organic N recycling but are conservative because they do not include production of other reduced, bioavailable N forms, such as urea.[50] Overall, CBAD values reflect the degree of NH_4^+ deprivation by the microbial community and provide insights about development of NH_4^+ limitation of CyanoHABs in eutrophic systems.

RESULTS AND DISCUSSION

CBAD, reflecting community NH_4^+ limitation or demand in eutrophic waters, is approximated here by observing differences between the potential ability of the microbial community to assimilate NH_4^+ (potential uptake) and actual community recycling rates (i.e., regeneration, or actual uptake rates;[15,23] Figure 1). Although CBAD, like biological oxygen demand (BOD), is expressed as a rate, it reflects a state or condition more accurately than a concentration in nature because it quantifies the "rate deficit". CBAD reflects the demand for NH_4^+ from the entire plankton community (e.g., phytoplankton, bacteria, archaea), including luxury consumption, whereas nutrient limitation often applies arbitrarily to specific taxa or, more loosely, to entire lakes or other aquatic systems.

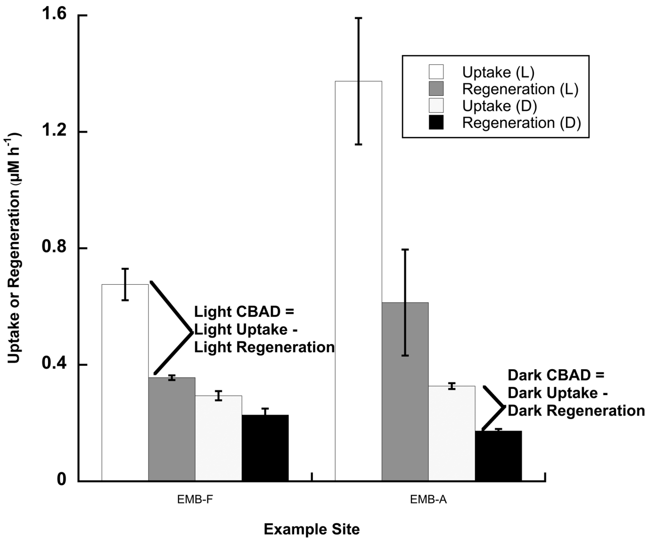

Figure 1. Example of CBAD calculations from NH_4^+ uptake and regeneration rates where light CBAD is the difference between uptake and regeneration in samples incubated under in situ light conditions and dark CBAD is the difference between uptake and regeneration in samples incubated in the dark. Example site is East Matagorda Bay.[20]

General evidence for NH_4^+ limitation of plankton is provided by comparing CBAD values from numerous systems, including

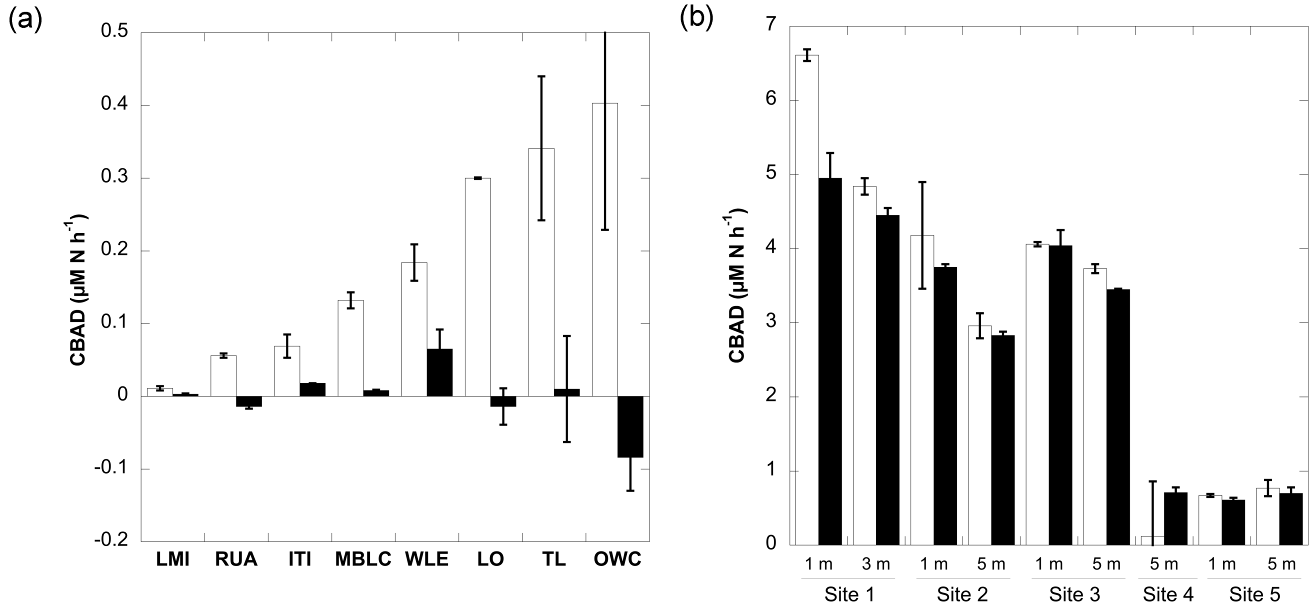

Figure 2. Community biological ammonium demand in a variety of eutrophic systems aquatic systems (A) and Lake Maracaibo in 1995 (B). White bars are light CBAD, black bars are dark CBAD. LMI: Lake Michigan, MI; RUA: Lake Rotorua, New Zealand; ITI: Lake Rotoiti, New Zealand; MBLC: Missisquoi Bay, Lake Champlain, Canada; WLE: Maumee Bay in western Lake Erie, OH; LO: Lake Okeechobee, USA; TL: Taihu Lake (China); OWC: Old Woman Creek, OH.

eutrophic lakes (Figure 2a). Highest CBAD values were observed in hypereutrophic Lake Maracaibo, which provided an extreme case to examine CBAD. Demand was much higher than supply, even with rapid recycling rates (Figure 2b). High NH_4^+ recycling rates in Lake Maracaibo[26] exemplify the effects of high system productivity and regional differences in N dynamics. Highest CBAD occurred near the sewage outflow in the Strait of Maracaibo (site 1). These values decreased with distance from the Strait but declined by 80% in southern Lake Maracaibo (sites 4 and 5;[26]) near the Catatombo River mouth (Figure 2B), perhaps due to light limitation. Not surprisingly, considering the tropical climate and degree of pollution, this system showed much higher rates of microbial NH_4^+ dynamics, including CBAD (particularly in regions affected by sewage and industrial inputs), than other lakes studied in more temperate and less eutrophic environments (e.g., Lake Michigan, Lake Rotorua, etc.).

Photic CBAD values, the absolute differences between light and dark CBAD (e.g., Figure 1), were normalized to chlorophyll a (Chl) to approximate algal biomass (Figure 3). The ratio of photic CBAD (μM h^{-1}) to Chl (mg L^{-1}) increased with increasing Chl (Figure 3), implying that organisms may be increasingly NH_4^+-deprived in eutrophic lakes as algal biomass increases.

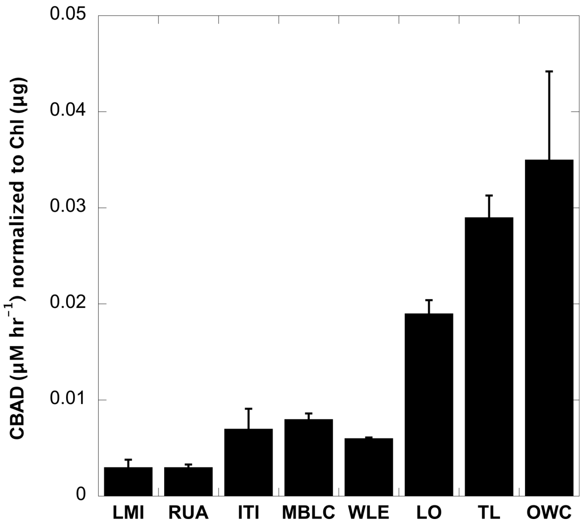

Figure 3. Regional averaged photic CBAD normalized to Chl. Site abbreviations are provided in Figure 2. Lake Maracaibo data was not included, as experiment incubation times were <4 h in Lake Maracaibo versus >16 h for other systems with lower rates, so dark CBAD values are not useful for calculating diurnal photic CBAD values.

This NH_4^+ deprivation emphasizes the importance of the internal supply of reduced N forms from rapid recycling, especially in lakes where NH_4^+ recycling rates exceed external loads. This pattern is observed commonly in eutrophic lakes, such as Missisquoi Bay, Lake Champlain, where the recycling N supply was about twice the external N load.[30]

Light, dark, and photic CBAD results for Maumee Bay (western Lake Erie, during nonbloom periods), central Lake Erie, and Meiliang Bay, Lake Taihu (Figure 4 and SI Figures 1, 2, and 4) show that microbial NH_4^+ demand is variable, regionally and temporally, even under nonbloom conditions. In particular, photic CBAD was highest near the tributary sources and decreased in more open waters in both Maumee Bay and Meiliang Bay (Figure 4). Rapid turnover of labile organic matter can occur at the river mouths, where dark CBAD values were actually negative, indicating that heterotrophic remineralization of NH_4^+ was faster than uptake rates in the dark (SI Figures 1 and 2). These sites also have the highest photic CBAD values, the absolute difference between light and dark CBAD. This phenomenon was not observed at open lake stations away from tributary nutrient inputs. In contrast to Meiliang Bay, East Taihu plankton (SI Figure 1) had low light CBAD and negative dark CBAD values, indicating that recycled N was sufficient to meet the pelagic community demand (or that the community was saturated). However, this area of the lake is dominated by submerged, rooted macrophytes,[38] with

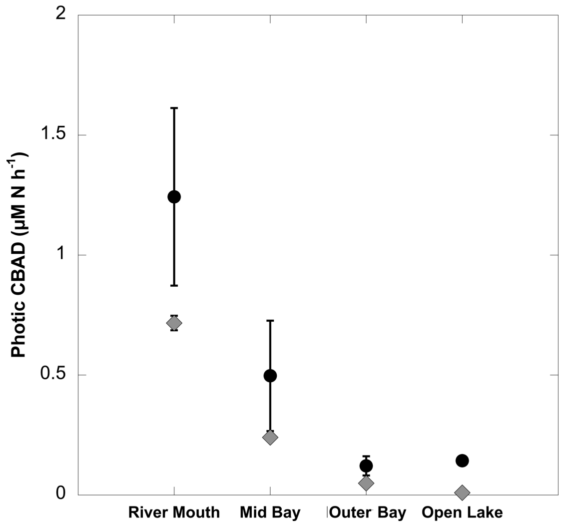

Figure 4. Comparison of photic CBAD from river mouths to the outer bays and adjacent open lake regions in Meiliang Bay, Lake Taihu (May 2004; black circles), and Maumee Bay, Lake Erie (July 2005; gray diamonds).

relatively low phytoplankton biomass in the pelagic zone. The highest degree of NH_4^+ depletion in Maumee Bay, as indicated by light CBAD values of 0.40 μM h^{-1} and photic CBAD values of about 0.75 μM h^{-1} (Figure 4, SI Figure 2), occurred in river mouth regions, compared to ca. 0.02 μM h^{-1} at the station furthest from the river mouth (Figure 4, SI Figures 1 and 2).

A second series of Lake Erie sites, sampled in September 2005, consisted of one site within 10 km offshore of Cleveland, OH, and four deeper sites in the central basin of Lake Erie (SI Figure 3). Water collected from the shallower two sites had light CBAD values of ca. 0.080 (site HE) and 0.025 (site 958) μM h^{-1}, respectively. Light CBAD values from deeper sites were not significant, but photic CBAD, reflecting negative CBAD values in the dark, were measurable (SI Figure 4). Light CBAD values for offshore regions of Lake Erie (SI Figure 4) were at or near zero, even though NH_4^+ cycling rates were measurable, indicating that recycling rates were sufficient to meet NH_4^+ demand. Dark CBAD values were negative, resulting in measurable, but not dramatic, photic CBAD offshore. We speculate that nutrient cycling processes in offshore Lake Erie surface waters were decoupled by depth and stratification from those in the bottom waters/sediments, where conditions were presumably suitable for denitrification. Much of the labile organic N degradation may have occurred in the oxygenated settling particles before they reached the sediments.[51]

The CBAD concept and related results address our central questions about the importance of NH_4^+ for the development and proliferation of CyanoHABs. We present a conceptual model (Figure 5) based on the CBAD data presented here and from previous N dynamics results (e.g., refs 10, 11, 21, 30, 52, and 53). The data and model suggest that labile organic N formation (algal biomass) and recycling transform N to reduced N forms (e.g., NH_4^+ and urea). These processes, in combination with system morphology and water movement from tributaries, are critical to the development and maintenance of non-N fixing CyanoHABs, as tributary and other inputs are integrated into lake biogeochemical dynamics.

Comparing light, dark, and/or photic CBAD in numerous aquatic ecosystems (SI Figures 2–4) suggests that microbial populations (including phytoplankton) were NH_4^+ deficient consistently. CyanoHABs show a strong preference for reduced N compounds.[42−45] We speculate that they help "pump" N out of the system by assimilating NH_4^+ into biomass, with delivery of the biomass to sediments or hypoxic waters, with eventual denitrification, burial, or export of the N.[52] These exports and losses in eutrophic lakes are typically not matched by N_2 fixation; hence, they perpetuate system N limitation.[11] Thus, labile organic matter cycled during CyanoHABs also may drive heterotrophic oxygen loss and denitrification in shallow water and sediments.[54] Coupled nitrification/denitrification increases overall NH_4^+ removal and decreases N/P ratios, despite high tributary nutrient inputs and cycling rates,[55] which we hypothesize would be subsequently reflected in higher CBAD values.

The internal formation and metabolism/degradation of particulate and dissolved labile organic N, driven by assimilation of internal and external nutrients, are critical model components. Regardless of the N forms introduced by tributary or other sources, rapid N uptake and transformations convert much of the non-NH_4^+-N to NH_4^+ and other reduced N forms (e.g., urea) via remineralization and excretion. Microbial metabolism of labile organic N provides NH_4^+ (and other reduced N forms), which are assimilated and recycled or oxidized to nitrates (supporting denitrification). Biochemical energy flow from these and other labile compounds (e.g., algal exudates)[56] consume O_2 through metabolism, driving environmental conditions toward those suitable for denitrification (Figure 5). Increased N incorporation into biomass may enhance N losses through burial, but fresh organic N recycles rapidly.[57] Denitrification rates relate to carbon respiration[30,54,58] and vary among systems.[59,60]

Microcystis typically blooms during seasonal temperature maxima (Table 2). These favorable water column conditions act in concert with high overall N and P supply rates from external, occurring mostly in spring, and internal sources. The microbial food web, a major source of recycled nutrients in most aquatic ecosystems,[61] is active during summer in productive freshwater ecosystems.[34,62−64]

The nutrient status of any aquatic community must be considered in the context of spatial and seasonal phytoplankton dynamics rather than as a permanent property of productive

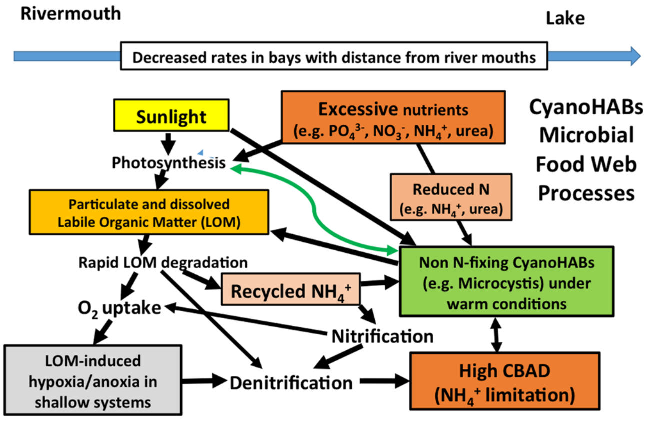

Figure 5. Conceptual model of how excessive tributary nutrient inputs lead to CyanoHABs and community NH_4^+ limitation. Internal cycling of labile organic matter (LOM) enhances production of reduced N forms for CyanoHABs and NH_4^+ limitation and promotes sediment nitrification/denitrification.

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Table 2. Nonparametric Spearman's ρ Correlations for the Respective Forms of Calculated CBAD with Temperature in Several Lakes (Maracaibo, Michigan, Taihu, and Erie, including Old Woman Creek, Missisquoi Bay, Lake Rotorua, and Lake Rotoiti), over a Wide Range of Temperatures and Locations[a]

variable	Spearman ρ	prob > $\lvert\rho\rvert$
light CBAD	0.775	<0.001
dark CBAD	0.534	<0.001
photic CBAD	0.486	<0.001
light CBAD/Chl	0.627	<0.001
dark CBAD/Chl	0.270	0.005
photic CBAD/Chl	0.529	<0.001

[a] See Table 1 for details.

lakes. An algal bloom represents an accumulation of phytoplankton biomass. While CyanoHABs are the product of excessive external nutrient loads and rapid internal recycling under suitable temperature conditions (Table 2), NH_4^+ limitation of CyanoHAB results from, rather than causes, algal blooms. Whereas division rates of individual phytoplankton cells are regulated primarily by dissolved minerals and physics (light, temperature), their population biomass is also subjected to top-down regulation by grazing[65] and virus attack.[63,66] If grazers (or viruses) fail to control a specific phytoplankton group initially, algal biomass will accumulate, even while cell division rates decrease due to gradually intensifying competition for nutrients. Elevated NH_4^+ demand occurs in many eutrophic ecosystems (e.g., refs 15, 23, and 67). Spatial and seasonal phytoplankton dynamics are important to N transformations (e.g., ref 68) and CBAD patterns in productive lakes.

The validity of our conceptual model (Figure 5) is illustrated with data from the 2015 seasonal CyanoHAB pattern in western Lake Erie. Incubations were conducted at three sites in Maumee Bay (SI Figure 3) before, during, and after a *Microcystis* CyanoHAB in summer 2015. The objectives were to (1) examine whether the conceptual model (Figure 5) was relevant to a specific CyanoHAB event and (2) determine whether CBAD would increase during a CyanoHAB. Specific evidence of NH_4^+ deprivation was provided by extreme CBAD values during August, when *Microcystis* biomass peaked. During nonbloom periods (June, July, and September), light CBAD values ranged from 0.062 to 0.460 μM h^{-1}, and dark CBAD values ranged from 0.116 to 0.162 μM h^{-1}. By contrast, light CBAD values in Maumee Bay (sites 2 and 6) during the CyanoHAB in August ranged from 6.5 to 9.9 μM h^{-1} and were sometimes orders of magnitude higher than during nonbloom periods (Figure 6). Even Site WE4 (SI Figure 3), at the edge of the surface CyanoHAB and influenced more by the Detroit River, had near-surface and near-bottom CBAD values of 2.2 and 0.12 μM h^{-1}, respectively, during the August bloom. CyanoHABs likely contributed to CBAD by competing successfully for NH_4^+ from external and recycling sources. Maumee Bay CBAD values after the bloom were comparable to prebloom levels (Figure 6). The high CBAD during CyanoHABs is explained by the relationship between temperature and CBAD (Table 2). Significant correlations were observed between temperature and light CBAD, dark CBAD, and photic CBAD (p < 0.001 for each; Table 2). These correlations are expected because CyanoHABs thrive at high temperatures.[69] The relationship between CBAD/Chl to temperature were also significant (<0.001), but that of dark CBAD/Chl to temperature (0.005) was not as strong. These data are consistent with the idea that CBAD reflects NH_4^+ limitation of non-N_2-fixing CyanoHABs in warm waters.

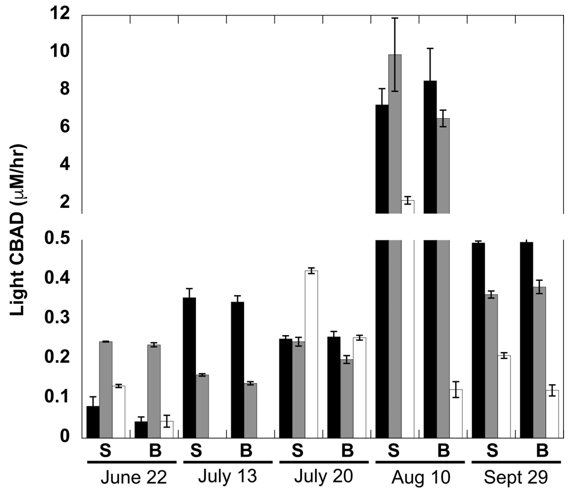

Figure 6. Temporal changes in light CBAD in surface (S) and bottom (B) waters at stations 6 (black bars), 2 (gray bars), and 4 (white bars) in 2015. Stations 6 and 2 were within the main CyanoHAB in Maumee Bay, whereas station 4 was near the limit of the surface bloom.

Spatial and temporal studies of NH_4^+ cycling and demand provide keys to understanding CyanoHAB dynamics. CBAD provides an integrated approach to characterizing the degree of NH_4^+ limitation of the plankton community. A microbial food web explanation for the extremely high CBAD during non-N-fixing CyanoHABs is that high affinity for NH_4^+ and rapid growth rates of the CyanoHAB algae outpace the lower reproduction rates of their predators. Thus, the CyanoHAB itself leads to nutrient limitation and death of component organisms (e.g., by autolysis after viral attack), which in turn provides labile organic matter. Large ecosystems, such as the Great Lakes and Lake Taihu, cannot be characterized as limited by only P or N based on monitoring nutrient concentrations (e.g., ref 70), especially when sampling locations are far from inputs and centers of biomass. High CBAD values under extreme CyanoHAB conditions suggest that differential trophic dynamics and rapid N cycling cause NH_4^+ limitation or colimitation of the CyanoHAB. These results emphasize the need for dual nutrient control (N and P) because relative nutrient limitation or colimitation can change frequently in time and space.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.6b06296.

P and N comparison, stoichiometric explanation, methodological details, temperature table for sampling sites, Lake Taihu CBAD figure, Maumee Bay CBAD figure, Lake Erie site map, Lake Erie CBAD figure, and list of references (PDF)

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Notes

The authors declare no competing financial interest.

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