Measuring nitrogen transformation rates in large, eutrophic lakes: challenges, solutions, and future needs

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The planet has a fever

The green line represents the upper safe limit of each of the earth’s 10 life-sustaining biophysical systems. Where “mercury” has risen above that line, humanity has already transgressed the boundary, risking potentially irreversible “tipping points.”


Rockstrom et al. 2009
The planet has a fever

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Slide Courtesy Bob Howarth

Rockstrom et al. 2009
Global trends in nitrogen use per year

(Erisman et al. 2015)
The paradigm:

Freshwater systems are phosphorus limited because nitrogen deficiencies are corrected by nitrogen fixation.

Data from Schindler et al. 2008 and Paterson et al. 2011.

Nitrogen fixation was sufficient to allow biomass to continue to be produced in proportion to phosphorus (Schindler et al. 2008).

Chl only related to P load when N also added, and Chl lower with P only than predicted from N+P.

$r^2 = 0.99$, $p = 0.0067$.
N- and Co-Limitation Isn’t Just For Bottles (or Lake 227)

(Paerl et al. 2016)
N Form Matters, Too

(McCarthy et al. 2009)

Large spread at high NH4:NOx

\[ y = 0.1996x + 0.2193 \quad R^2 = 0.4934 \]

\[ y = -0.1298x + 0.4864 \quad R^2 = 0.3180 \]
N Form Matters, Too

(and yet....quoted from L&O paper published in 2015...authors not identified to protect the guilty)

“Nitrate (NO3) can be used alone as an estimate of bioavailable nitrogen (N) in the Great Lakes...It should be noted that the high concentrations of nitrate are protective against cyanobacterial blooms.”

“In fact, ammonium (NH4+) and dissolved organic nitrogen concentrations...have remained relatively low, typically below detection limits; therefore, total nitrogen (TN) flux is dominated by NO3-...”
Nitrogen Control in Cyanobacteria

ntcA

“encodes a positive regulator of genes subject to negative ammonium control”

(Vega-Palas et al. 1990; Luque et al. 1994)

Expression required for all non-NH4-N metabolism, including N fixation (and metals required for heterocyte differentiation, and many other C, N, and P algal functions)
Nitrogen Control in Cyanobacteria

ntcA

“not transcripted until ammonium concentration is < 1 μM”

(Lindell & Post 2001)

Thus, all N metabolism in cyanobacteria is under the direct control of ammonium (Herrero et al. 2001)
Nitrogen Control in Cyanobacteria

ntcA

“transcription of the mcyABCDEFGHIJ gene cluster” (microcystin production genes)

(Ginn et al. 2010)

Thus, all N metabolism AND (microcystin) toxicity in cyanobacteria is under the direct control of ammonium (Scott et al. 2013).
Nitrogen Control in Cyanobacteria

The Bottom Line

N form matters, and these studies provide the genetic basis to explain why field and lab studies have shown differences in growth and competition by phytoplankton groups for different N forms (e.g., cyanos v. diatoms, and reduced N v. oxidized N (i.e., NH4 v. NO3) v. N fixation)

TUG OF WAR: THE COMPETING ROLES OF SEDIMENTS IN EXACERBATING AND MITIGATING EUTROPHICATION IN LAKES FROM A NITROGEN PERSPECTIVE

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Nitrogen cycling processes include:

- **N\textsubscript{2} fixation**
- **Diazotrophic assimilatory nitrogen reduction (DNRA)**
- **Anammox**
- **Nitrification**
- **Denitrification**

Processes involved include:

- **OM** (Organic Matter)
- **NH\textsubscript{4}^+** (Ammonium)
- **NO\textsubscript{3}^-** (Nitrate)

The diagram illustrates the flow of nitrogen species between water, sediment, and the atmosphere.
*Collect intact sediment cores and near-bottom water for continuous-flow incubations (with and without $^{15}$N) to measure SWI N fluxes and transformations.
Measurements

- Dissolved gases ($^{28}\text{N}_2$, $^{29}\text{N}_2$, $^{30}\text{N}_2$, $\text{O}_2$, $\text{Ar}$)
- MIMS (Kana et al. 1994, An et al. 2001)
Mean N removal Aug 2013 = 4.02 x 10^7 kg N yr^{-1}

Annual N Load = 3.98 x 10^7 kg N yr^{-1} (Taihu Basin Authority 2014; mean for 2007-2014; underestimate?)

Based on Aug 2013 rates, N_2 production removes 101% of annual N load

Based on rates measured in Fall 2002, Jan 2004, and May 2004 = 66% (Paerl et al. 2011)

Summer has higher N removal rates than other seasons, likely contributing to N limitation (Xu et al. 2010, Paerl et al. 2011)
Objective: Determine the relative contributions of denitrification to system N removal (N sink) vs. N recycling via DNRA (N link)

Conclusions:

(1) Denitrification can remove most (or all) of the external N load, and there’s little or no N fixation

(2) Despite this, non-N-fixing cyanobacteria blooms persist, often for 10+ months
If denitrification removes most (or all+) of external N loads, what fuels the annual non-N-fixing cyanobacteria blooms?

(1) SWI NH4 efflux exceeds external N inputs by a factor of 1.4 (legacy N?)

(2) The phytoplankton community is dominated by *Microcystis*, a non-N-fixer

(3) Non-N-fixers are highly competitive for reduced N forms (i.e., NH4 and urea; Blomqvist et al. 1994)
Ammonium regeneration in the water column supplies most of the ammonium demand in Taihu Lake (China)

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Funding: NSF-Dimensions in Biodiversity
Objective

Determine how much ammonium is regenerated in the water column relative to the sediments and external N inputs.
Bacteria and phytoplankton

Zooplankton/Mixotrophs

Uptake

Regeneration

$\text{NH}_4^+$
Bacteria and phytoplankton

\[ ^{15}\text{NH}_4^+ \]

Zooplankton/Mixotrophs

\[ ^{14}\text{NH}_4^+ \]

NH\(_4^+\) pool becomes diluted ("lighter")
NH4 WC regeneration Aug 2013 = $1.44 \times 10^8$ kg N yr$^{-1}$

NH4 WC potential uptake Aug 2013 = $1.81 \times 10^8$ kg N yr$^{-1}$

Sediment NH4 release Aug 2013 = $6.37 \times 10^7$ kg N yr$^{-1}$

Based on Aug 2013 rates, NH4 regeneration in the water column exceeds N load by nearly 2.6-fold and sediment NH4 release by 2.3-fold (holds up for other years and seasons; Hampel et al. 2018)

Sediment NH4 release can supply 35% of NH4 demand

WC NH4 regeneration can supply 79% of NH4 demand
Objective: Determine how much ammonium is regenerated in the water column relative to the sediments and external inputs

Conclusions:

(1) NH4 regeneration in the water column is likely the primary source of N for primary producers (up to 80%)

(2) Sediment + water column NH4 regeneration is usually enough to satisfy summer NH4 demand, but not in all seasons or annually (Hampel et al. 2018)

(3) In-lake NH4 regeneration vastly exceeds external TN inputs (but actual N load is likely higher than reported)

(4) Lake eutrophication management is more than just controlling P and calling it ‘fixed’; e.g., Lake Erie; nutrient management!!!)
Scaling Up – How good are these extrapolations of spatially and temporally limited data to whole systems and annual estimates?

Internal N cycling is certainly important, but:

1. It’s time and labor intensive, expensive, and requires highly specialized expertise and equipment to measure.

2. Subject to bottle/isolation effects.

3. Sometimes requires substrate additions and molecular techniques ($$$) to distinguish mechanisms.

4. Latitudinal considerations (ice cover, etc.)
Scaling Up

Internal N cycling is certainly important, but:

1. It’s time and labor intensive, expensive, and requires highly specialized expertise and equipment to measure. To resolve this issue, more sampling and manpower is needed, which requires:

$$
$$

$$
$$
Scaling Up

Internal N cycling is certainly important, but:

2. Subject to bottle/isolation effects.
   
   A. Experimental, large volume mesocosms, artificial lakes, etc.
   
   B. Develop whole-lake techniques, similar to those sometimes used in rivers.
   
   C. Develop and modernize ecosystem models, which currently lack sufficient resolution for internal N cycling (but new progress!).
Scaling Up

Internal N cycling is certainly important, but:

2. Subject to bottle/isolation effects.

D. Most importantly, results from these “containerized” incubations are the same as those from whole system experiments (i.e., regeneration processes are often driving N metabolism in blooms, combination of N and P usually results in higher biomass than either does alone).
Internal N cycling is certainly important, but:

3. Sometimes requires substrate additions and molecular techniques ($$) to distinguish mechanisms.

Follow the genes! Gene expression is definitive for N cycling processes; samples are not hard to collect, but it’s expensive to analyze and sequence genetic material samples.

$$
Internal N cycling is certainly important, but:

4. Latitudinal considerations (ice cover, etc.)

Subtropical/tropical lakes ≠ temperate lakes

We know very little about what happens with the N cycle in systems that are mostly inaccessible in winter, but lots of data from subtropical and tropical lakes in ‘winter’. Huge data gap for characterizing under ice N cycling. All it takes is time and $$$.
Summary

Internal nutrient loads are not just a P story. Internal N cycling (fueled by external loads!) is the main source of N for biomass and toxin production in non-N-fixing cyanobacteria blooms.

At the same time, the N cycle provides the best defense against excessive N loading via denitrification. BUT, this defense loses efficiency as N loads increase (e.g., Mulholland et al. 2008; Gardner & McCarthy 2009).

In many cases, N loading reductions are critical to restore lakes afflicted by toxin-producing, non-N-fixing cyanobacteria.
Summary

There are valid critiques of containerized, mechanistic experiments, but the results inevitably are the same (and at least we can replicate).

But, we can improve our management tools by incorporating mechanistic rate data into modernized ecosystem models that include actual data from the system in question (i.e., not ‘ancient’ data on a process from the literature, often not even from a similar system).

‘Snapshot’ nutrient monitoring has value, but for N (and reactive P?), mechanistic turnover/recycling data is the only way to determine the true ‘availability’ of NH$_4^+$, which drives all cyanobacteria N metabolism (even for N fixers).
“We can’t solve 21st century problems with 20th century science” --- Bob Heath, 2014-05-28
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