

How does the microbiome influence *Microcystis* blooms? (N availability, H₂O₂ defense)



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Microcystis blooms threaten freshwaters globally – how are they able to thrive under different conditions?

Lake Taihu, China



Lake Victoria, Africa



Lake Erie, MI/OH, USA



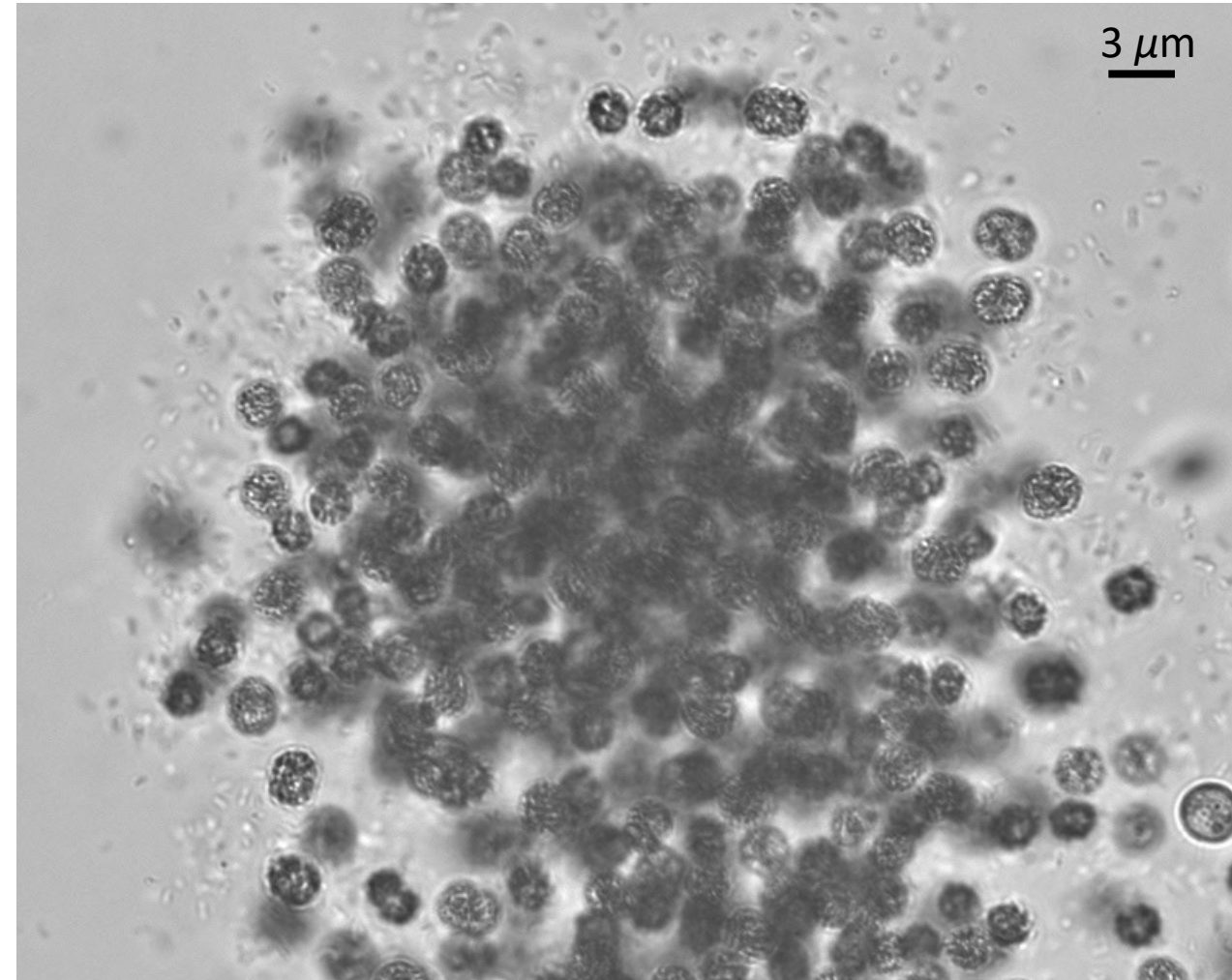
Klamath River, CA, USA



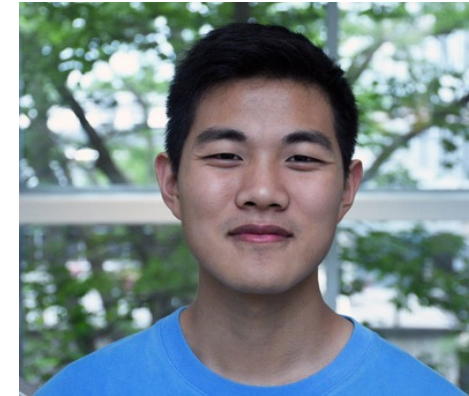
The *Microcystis* microbiome

Microcystis grows in colonies that harbor a dense microbiome

- “A hotspot for bacterial activity... a bacterial incubator”
(Worm & Sondergaard, 1998)
- What microbes are present?
 - specific or random?
 - variation across space, time, strain
- How do these microbes interact with *Microcystis*?
 - detoxification of H_2O_2
 - exchange of carbon, nutrients, vitamins



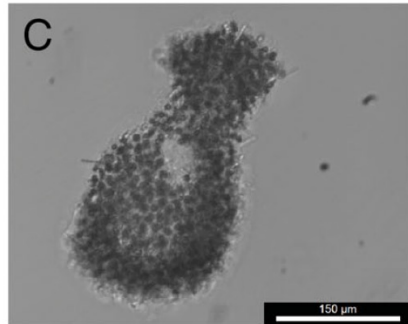
High-throughput isolation and sequencing of individual *Microcystis* colonies with droplet encapsulation



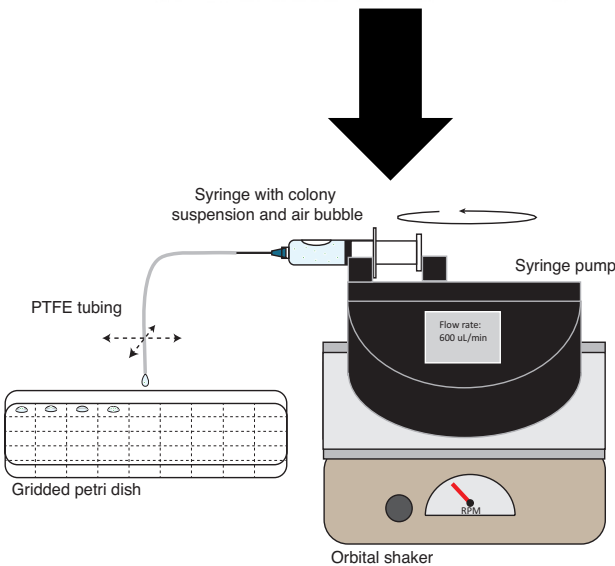
James Tan



Nina Lin



← wash & dilute



→ microdroplet encapsulation



one colony per droplet
Screened ~800 droplets

→
16S rRNA gene sequencing
~210 droplets

Community composition of individual *Microcystis* colonies

Microcystis colony + microbiome

Microcystis colonies harbor a microbiome that is distinct from bulk and particle-attached communities

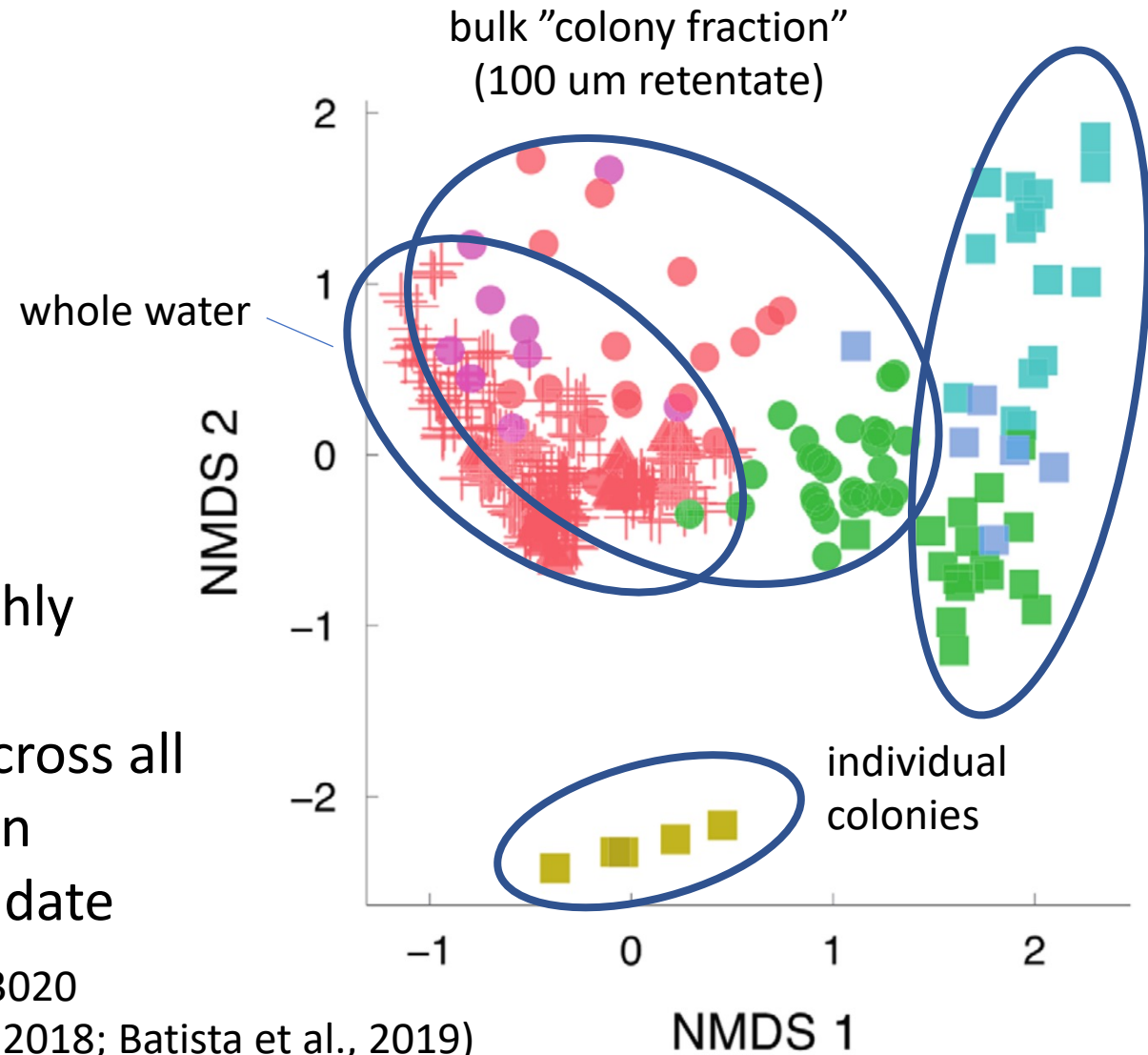
Sample Type

- Colony
- + Whole Water
- 100 μ m Retentate
- ▲ 105 μ m Filtered

Microcystis strain cluster

- A
- B
- C
- D
- E
- F

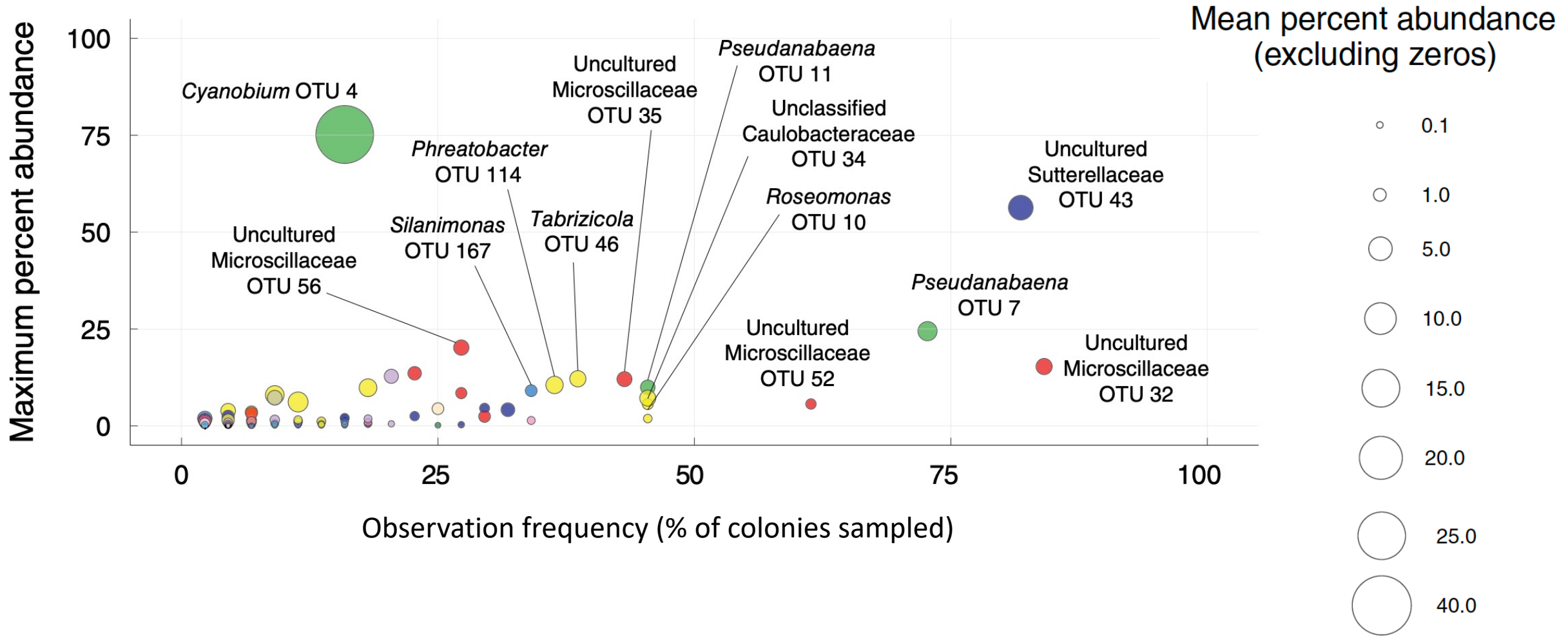
- The *Microcystis* microbiome is highly variable.
- only nine OTUs were conserved across all colonies on a specific date or strain
- Varies with *Microcystis* strain and date



Smith et al. *Environmental Microbiology* (2021) **23**: 3020

See also: Cai et al., 2014; Xu et al., 2018; Yang et al., 2018; Batista et al., 2019)

The *Microcystis* microbiome often contains certain taxa, but there are no "core" (universal) taxa



The microbiome influences *Microcystis*'s growth, toxin production, competitive interactions with other phytoplankton

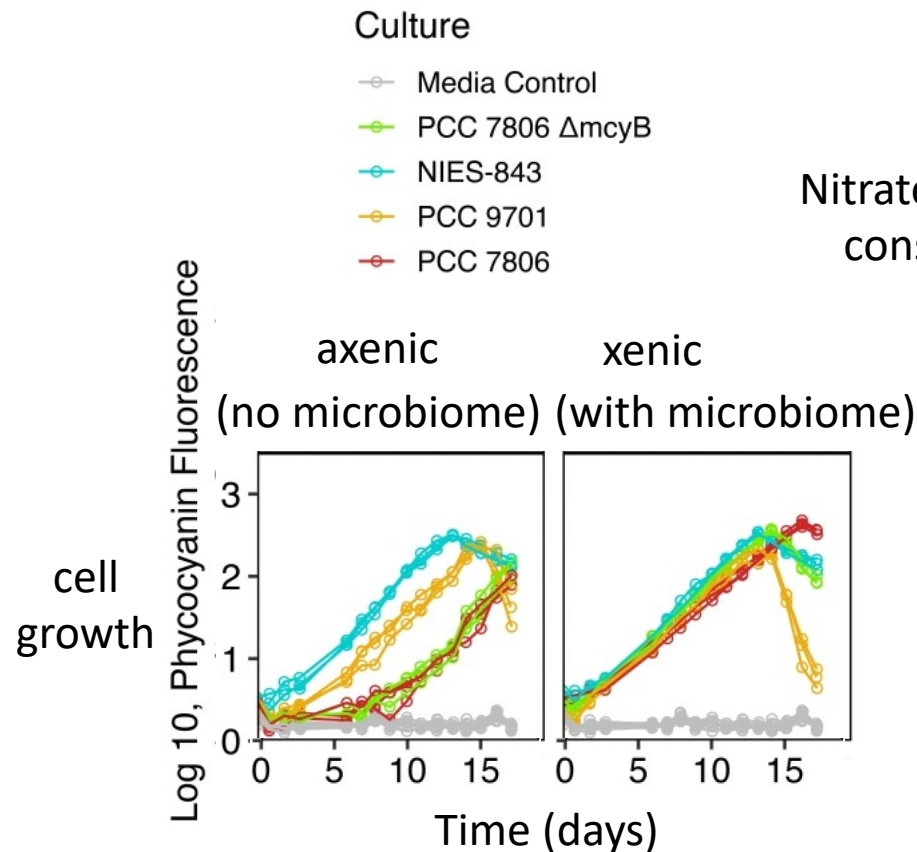
microbiome transplant experiments



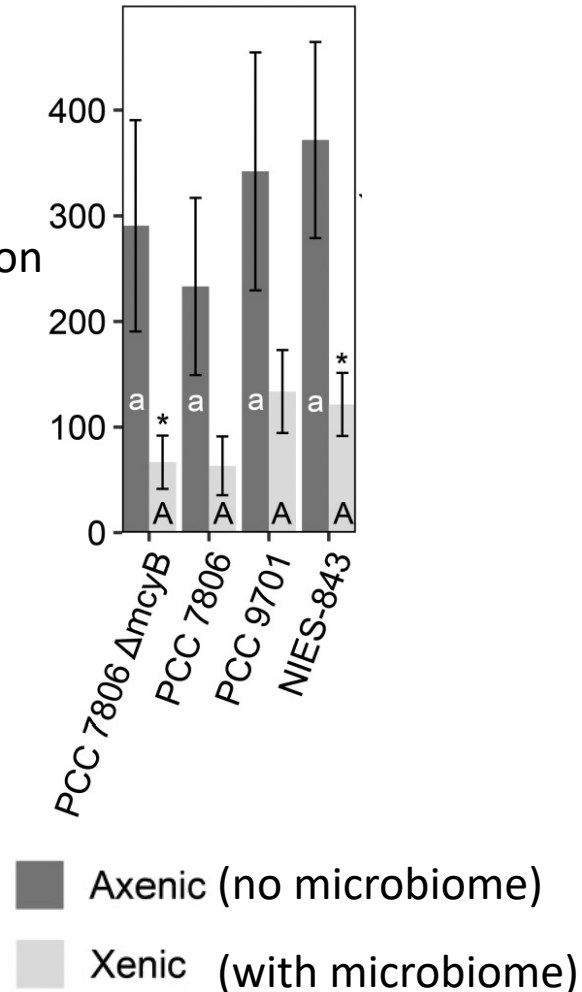
Baker et al, *mLife* (2023) 2: 401

Schmidt et al (2020) *Harmful Algae* 99: 101939

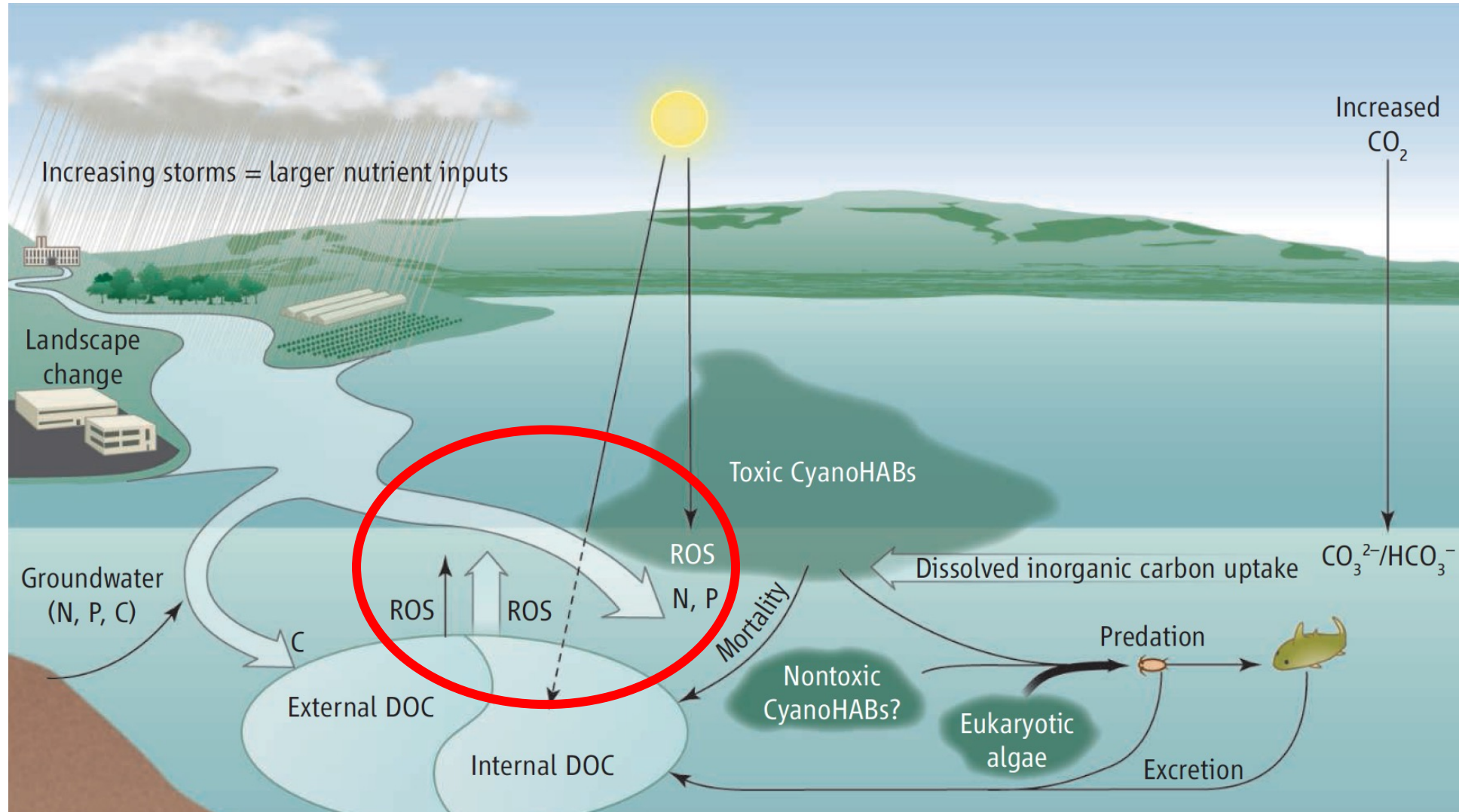
Li et al. (2023) *ISME J.* in review



Nitrate half-saturation constant, K_s (μg^{-1})



Does the microbiome protect *Microcystis* from reactive oxygen species (H_2O_2), influence strain composition & toxicity?

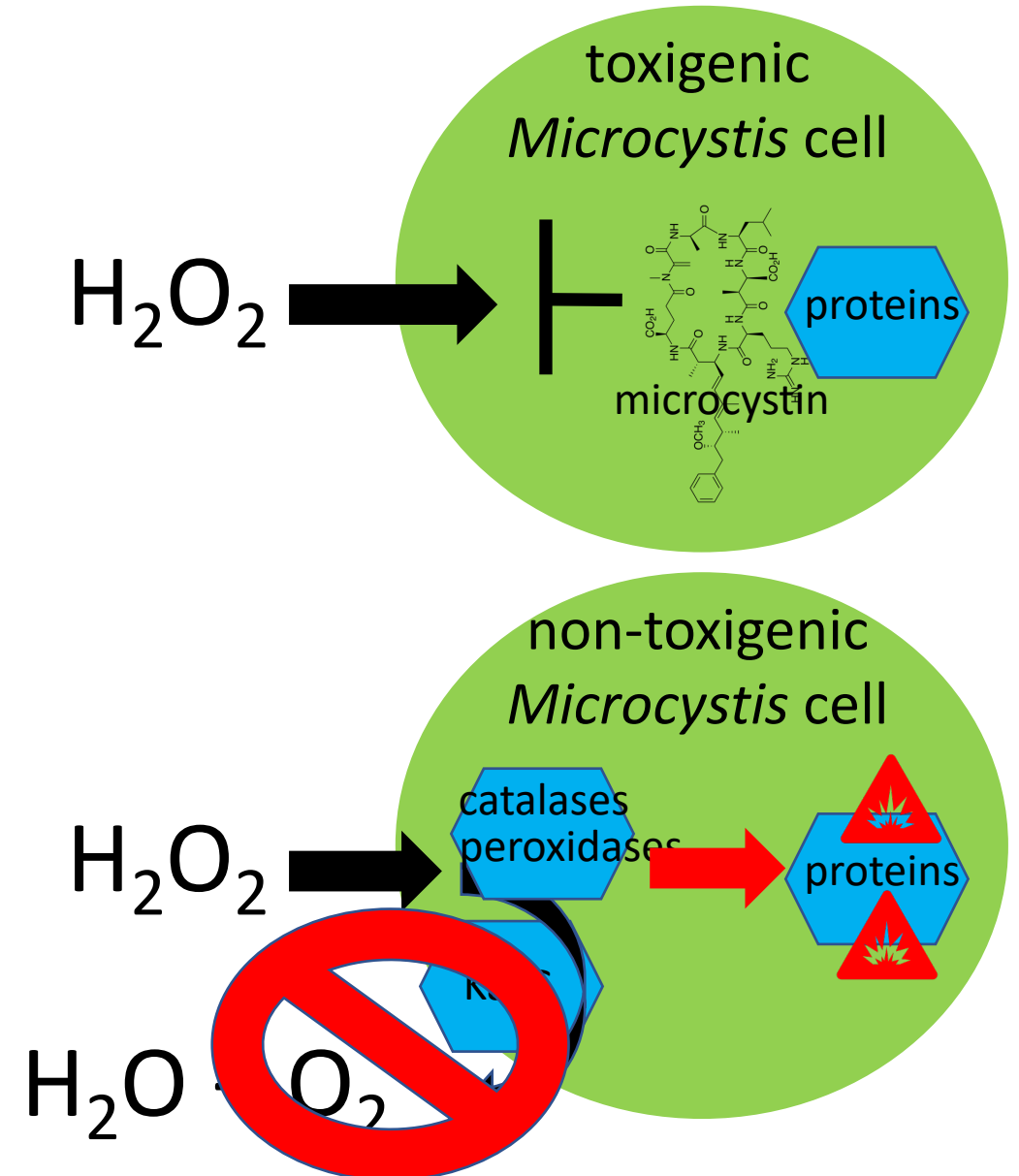
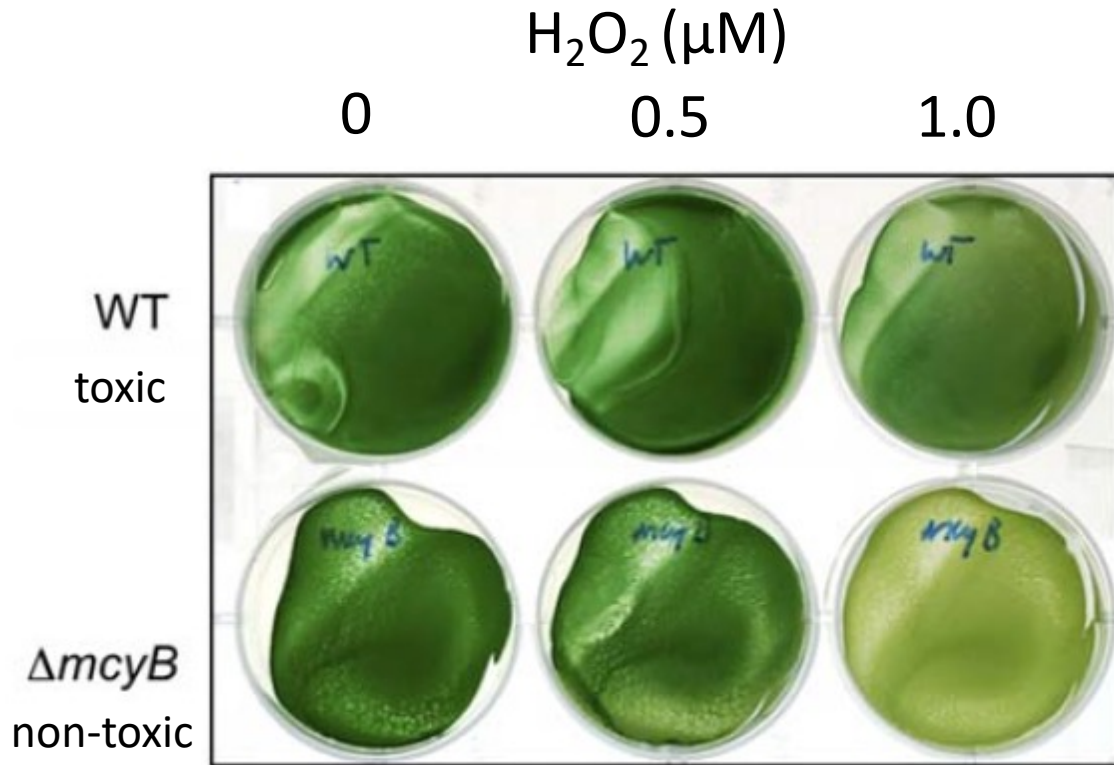


Microcystins protect *Microcystis* from reactive oxygen species such as H₂O₂

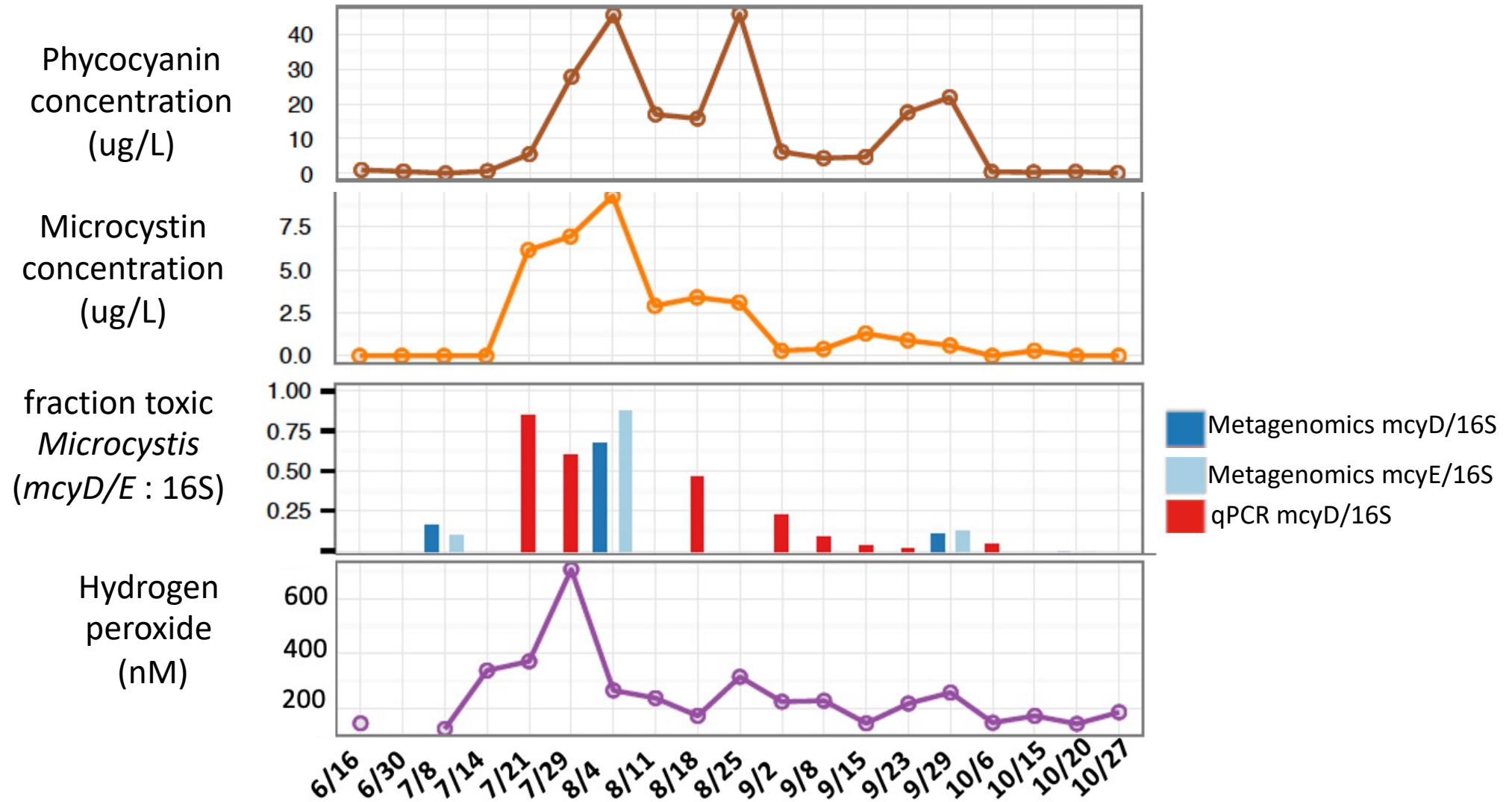
Microcystis lacks *katG*, suggesting that non-toxic strains may be susceptible to H₂O₂

The Cyanobacterial Hepatotoxin Microcystin Binds to Proteins and Increases the Fitness of *Microcystis* under Oxidative Stress Conditions

Yvonne Zilliges¹, Jan-Christoph Kehr², Sven Meissner², Keishi Ishida³, Stefan Mikkat⁴, Martin Hagemann⁵, Aaron Kaplan⁶, Thomas Börner⁷, Elke Dittmann^{2*}



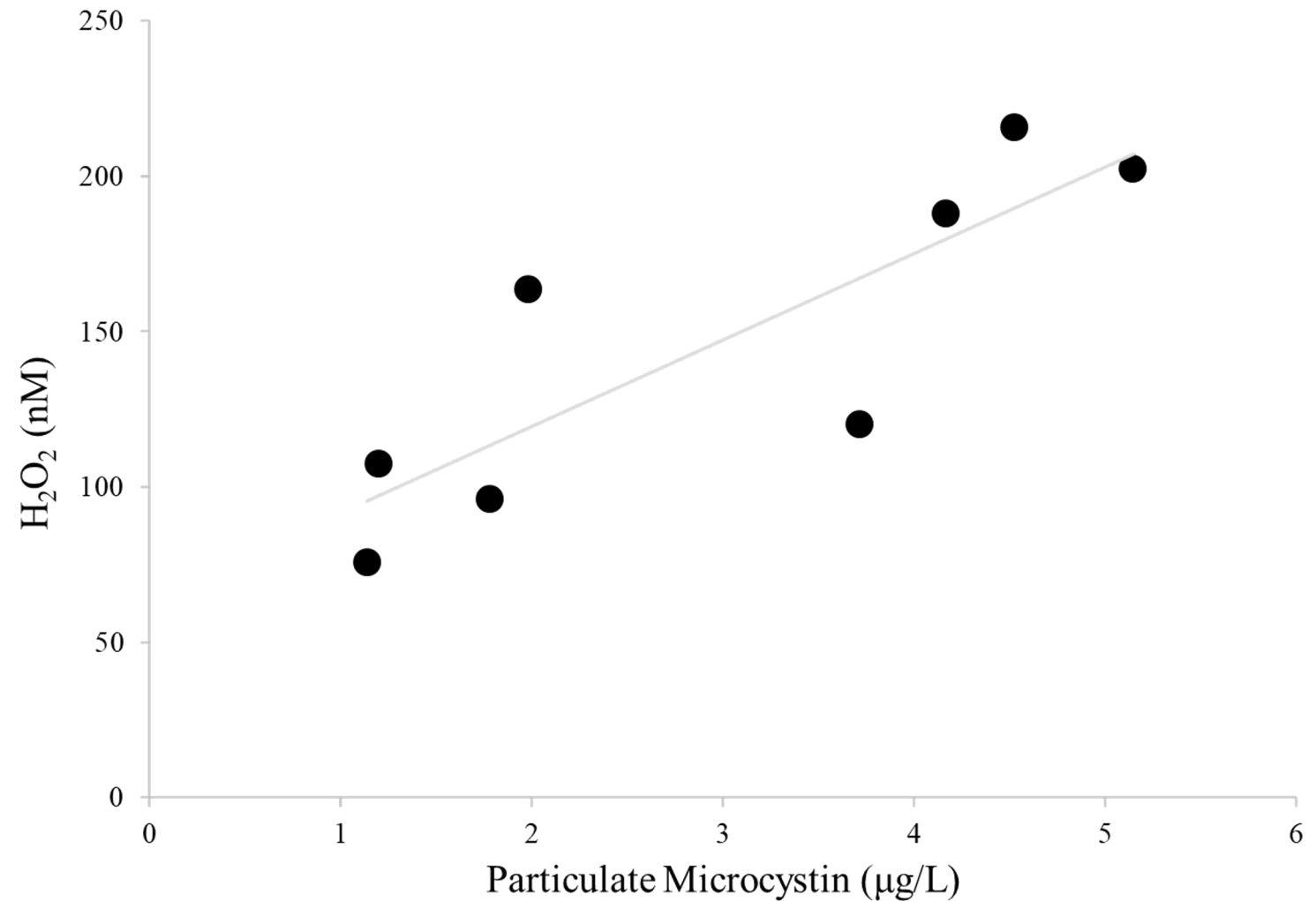
The *Microcystis* strain shift from toxigenic to non-toxigenic correlates with H₂O₂ concentrations



Average H_2O_2 and microcystin concentrations are significantly correlated in the western basin of Lake Erie



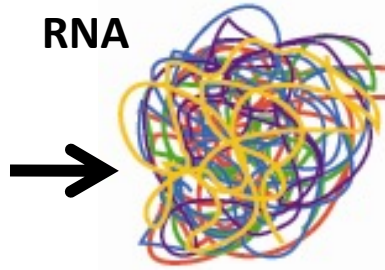
Rose Cory



Environmental omics allow us to track microbial communities and their functions in the field

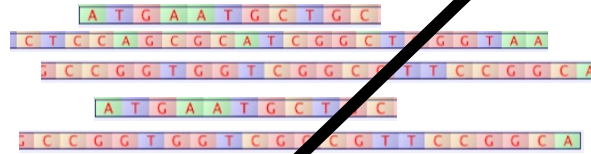


extract
DNA
RNA



random shotgun
sequencing

billions of DNA, RNA
sequences



map RNA
transcripts

genomic
assembly



thousands of contigs

What organisms are present?

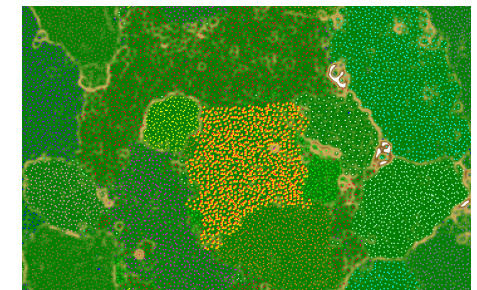
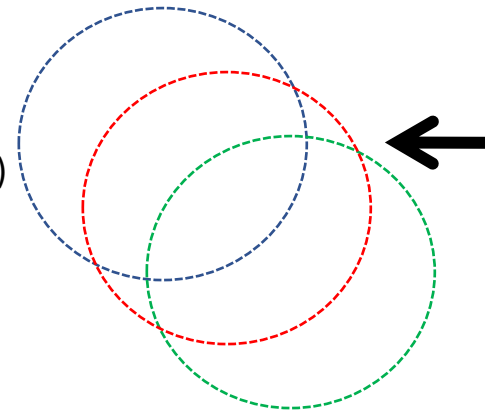
What are their nutrient & energy requirements? Stress defenses?

What toxins do they produce?

Which organisms, genes, metabolisms are active?

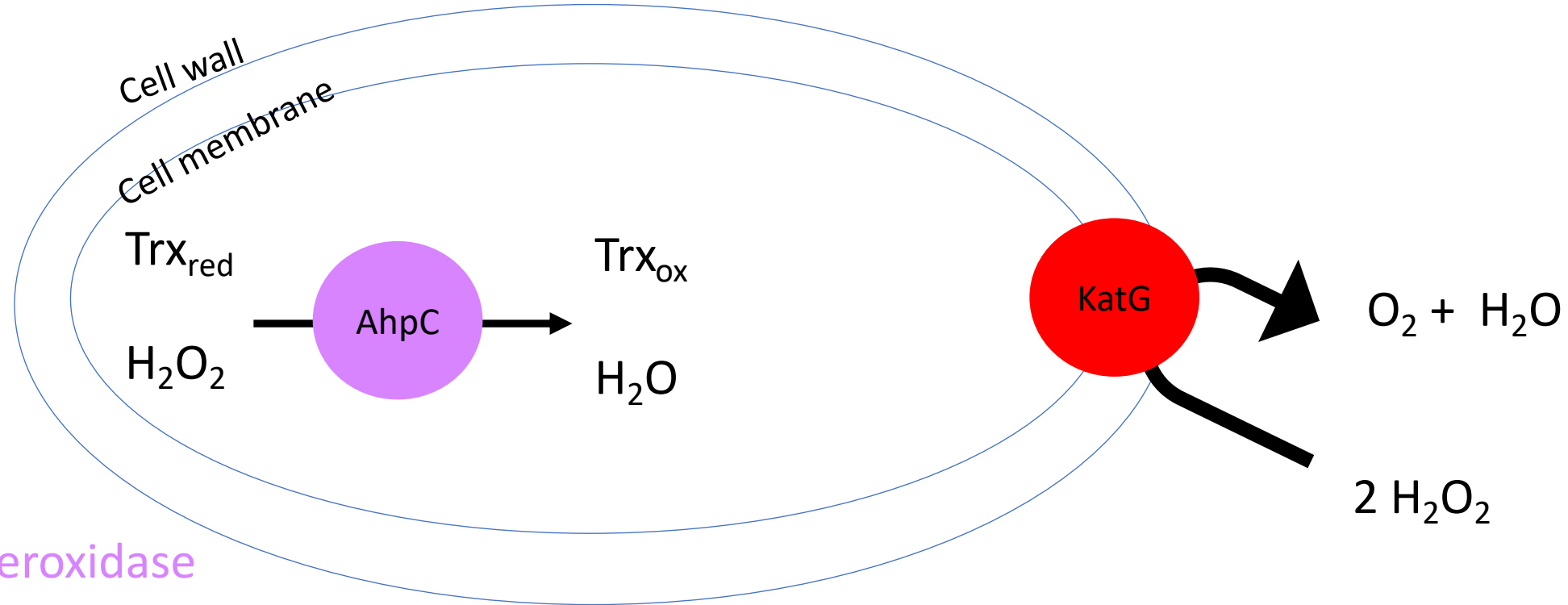
gene
annotation

complete genomes
(now up to thousands)



Genome binning

katG and *ahpC* are the most abundant genes for H₂O₂ decomposition in the community



AhpC = thioredoxin peroxidase

KatG = catalase

Cytoplasmic heme catalase (CAT)

Mn Catalase

Cytochrome c Peroxidase (CCP)

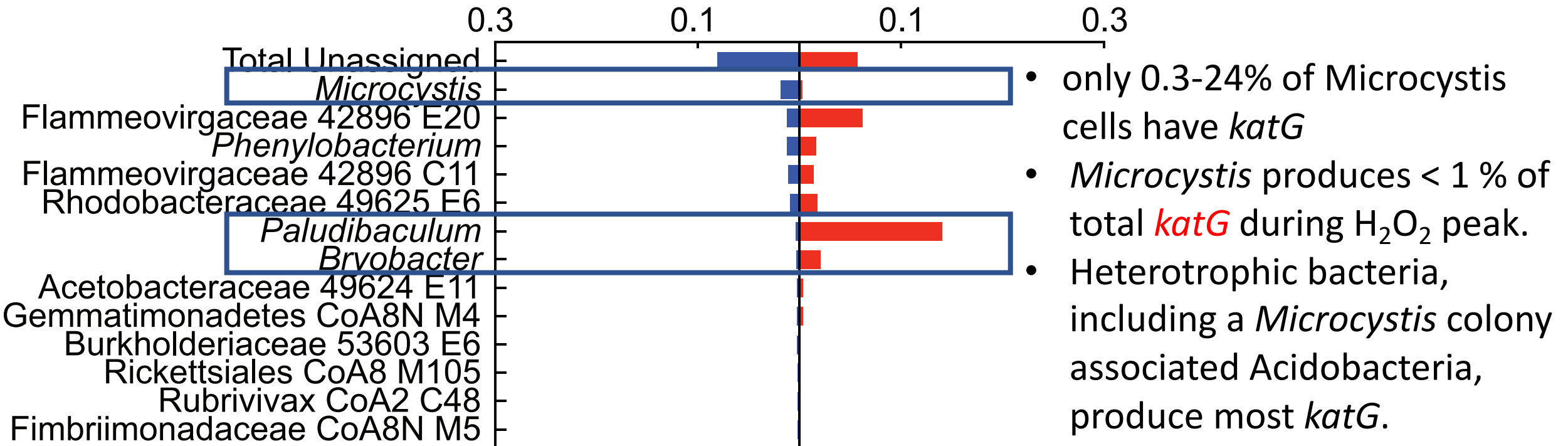
Cells that express *katG* are more resistant to high extracellular H₂O₂ conc.

Cells that lack *katG* are sensitive to extracellular H₂O₂ conc

katG transcripts (H₂O₂ degradation) are dominated by non-*Microcystis* bacteria

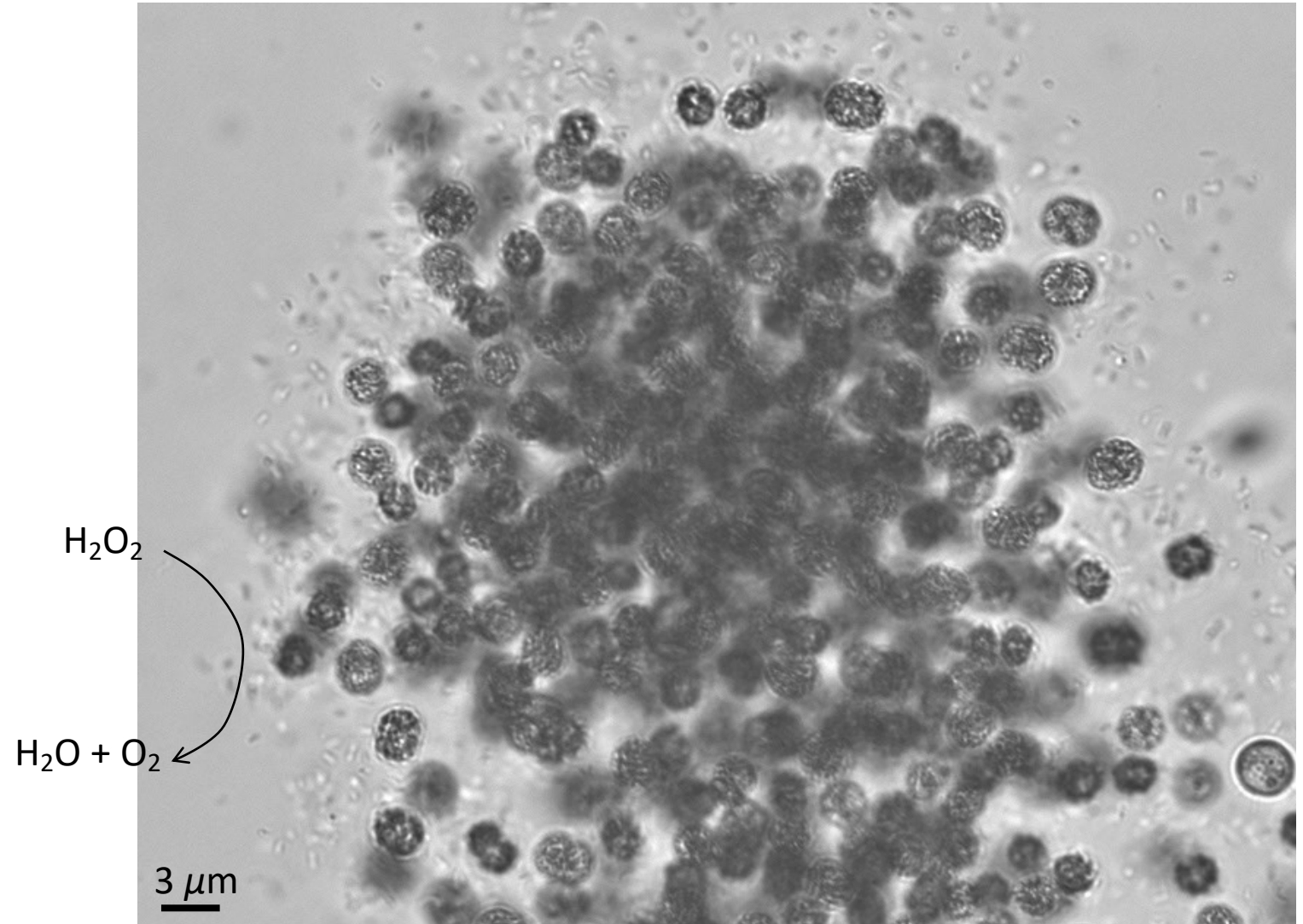
Relative Abundance (*katG:rpoB*)

■ Metagenome ■ Metatranscriptome



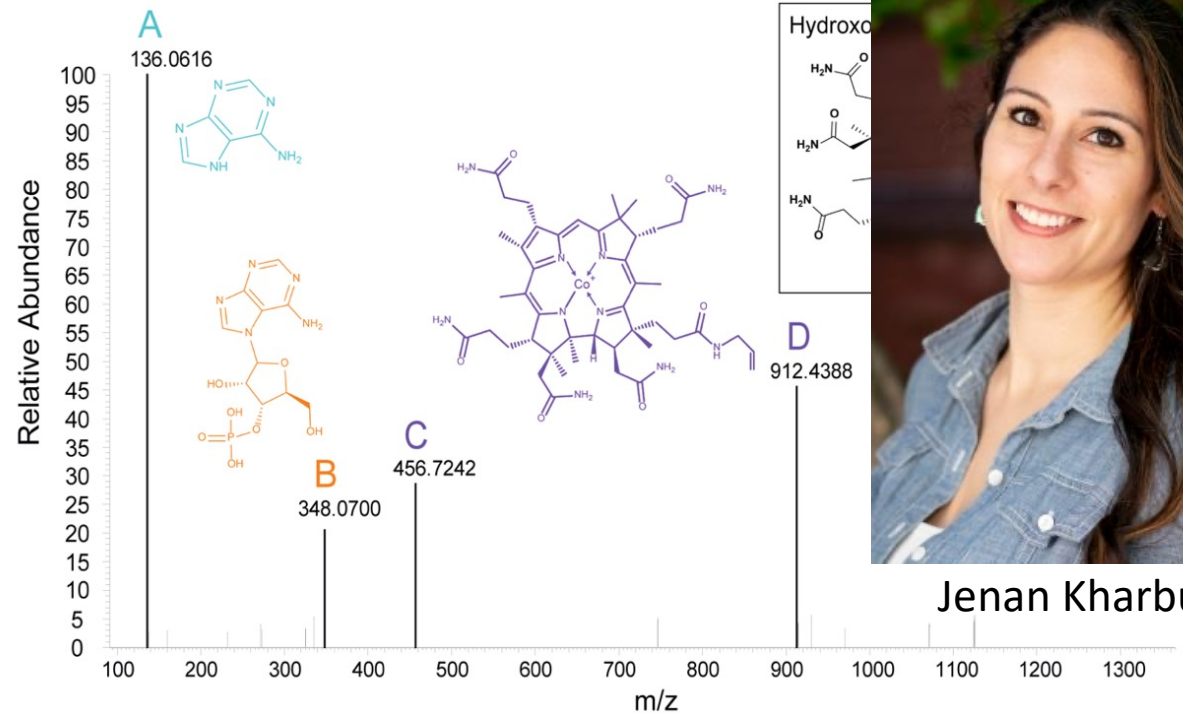
The microbiome likely benefits *Microcystis* by decomposing hydrogen peroxide (H_2O_2)

- *Microcystis* lacks catalase (*katG*), which decomposes H_2O_2 .
- "Helper" bacteria may protect *Microcystis* from H_2O_2 by decomposing it, reducing environmental H_2O_2 concentrations.
- **How do the helper bacteria benefit from this interaction? Look at the most highly expressed genes.**



Evidence that the Acidobacteria obtain vitamin B₁₂ from *Microcystis*

- Both Acidobacteria lack all genes for making vitamin B₁₂
- Both Acidobacteria have & express:
 - B₁₂-dependent enzymes
 - genes for B₁₂ remodeling
- The *Bryobacter* genome expresses genes for B₁₂ transport
- B₁₂ was detected in axenic cultures of *Microcystis aeruginosa*.
- *Microcystis* has B₁₂ biosynthesis pathway and expresses it in Lake Erie



Jenan Kharbush

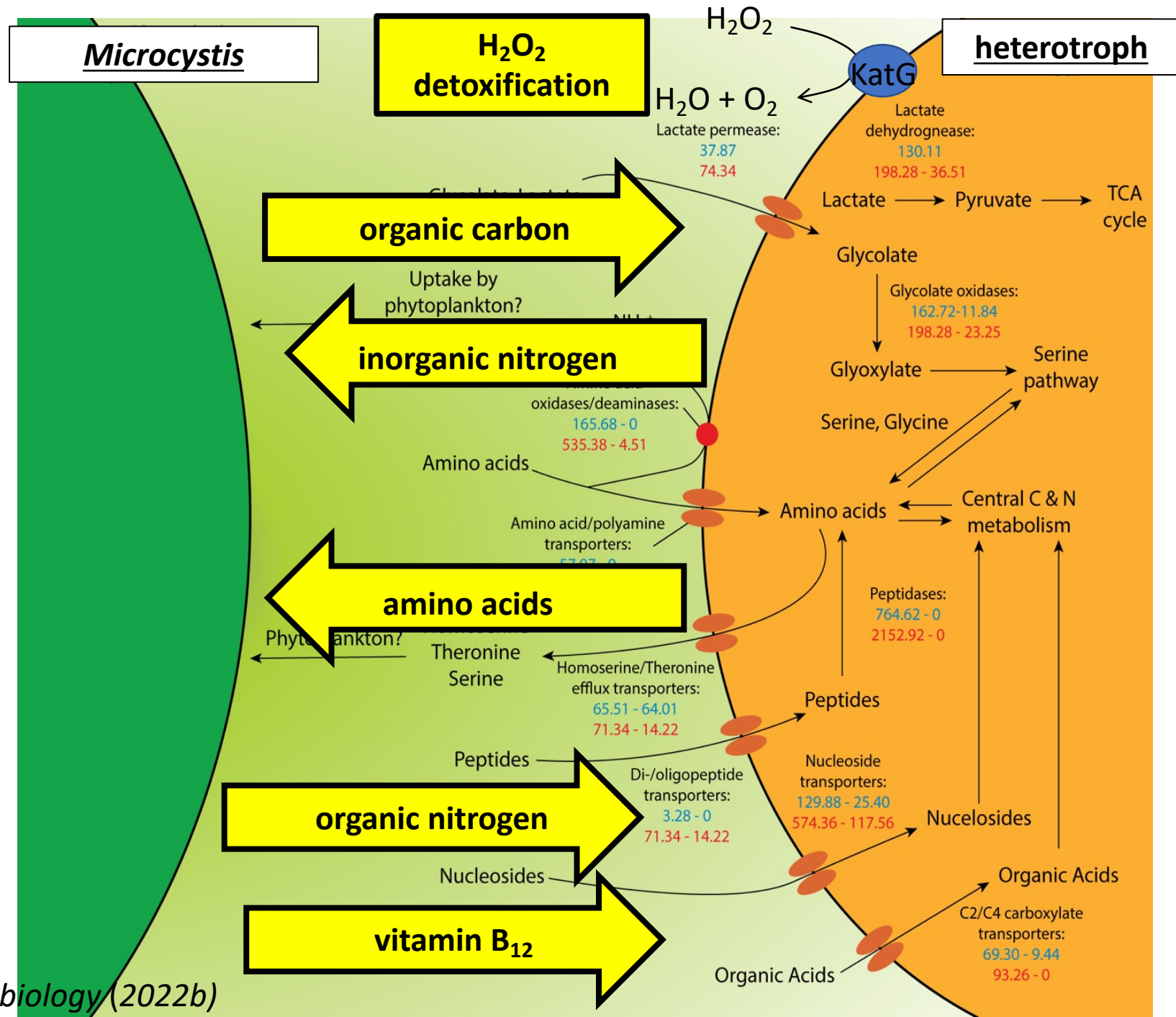
mass spectrum of hydroxopseudocobalamin from *Microcystis aeruginosa* PCC 7806 with the chemical structures for each fragment and for the entire molecule (black box)

Metatranscriptomic insights into uncultured *Microcystis*-associated Acidobacteria:

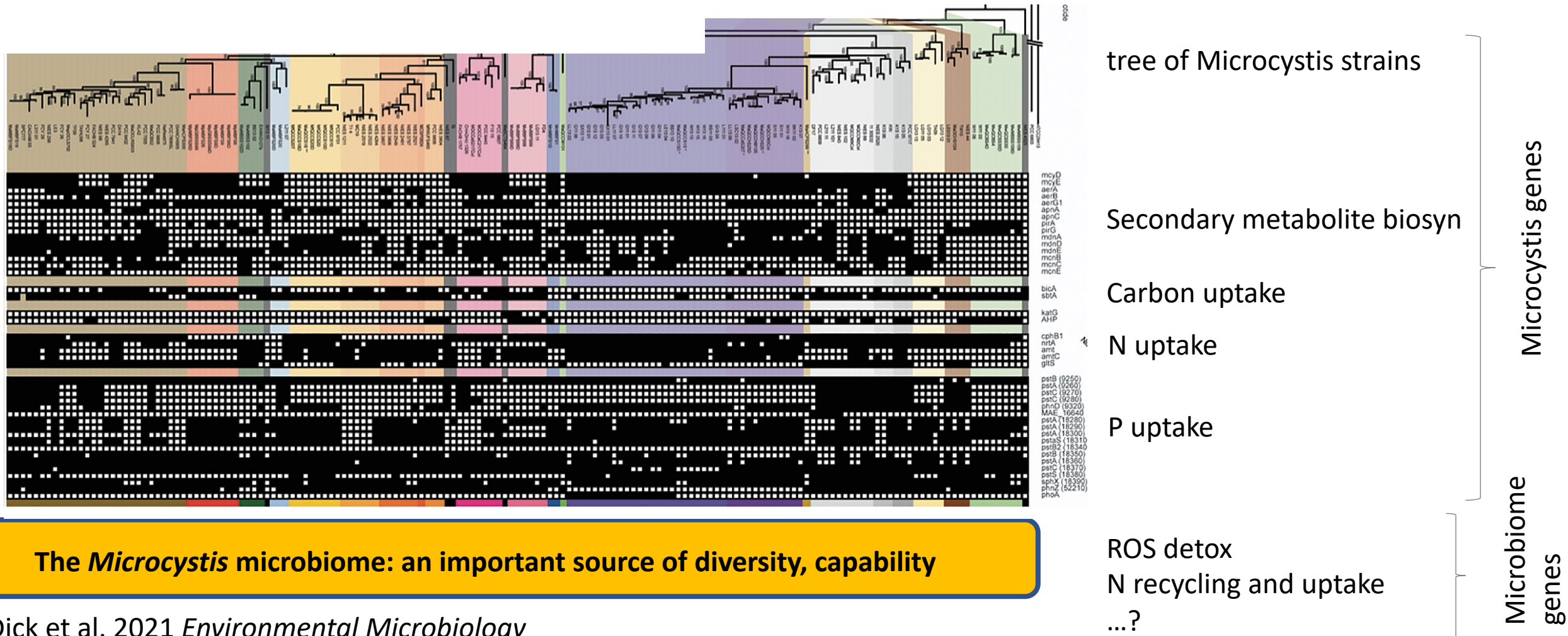
- Decomposition of H₂O₂ protects cyanobacteria from oxidative stress
- Use organic carbon and nitrogen from cyanobacteria (EPS, organic acids, amino acids, peptides, nucleosides)
- Regenerate ammonium – potentially crucial for N-limited phases of the bloom.
- Obtain vitamin B₁₂ from *Microcystis*

- mutualism
- This is just from 2 related symbionts!
- Present in Lake Taihu, other *Microcystis* blooms around the world.

Smith et al. *Applied and Environmental Microbiology* (2022b)



How does the *Microcystis* microbiome influence cyanobacterial blooms?



The *Microcystis* microbiome: an important source of diversity, capability

Thank you

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Robert Hein

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