***Title***: Stoichiometric Plasticity of Heterotrophic Bacteria in the Laurentian Great Lakes: The Impacts of Winter and Nutrient Concentration on Community Resilience

***Background:*** The Laurentian Great Lakes represent the largest freshwater ecosystem on Earth and support around 34 million people and their economies, while providing crucial habitat for biological communities [1]. Due to increased human populations, land use, and inefficient farming techniques, large influxes of nutrients and carbon (C) have been observed in the Great Lakes. The most impacted lakes are Erie and Michigan, receiving the highest nitrogen (N) (61.5 and 62.9 kt/y, respectively) and phosphorus (P) (2.4 and 2.3 kt/y, respectively), with the majority being runoff from manure and chemical fertilizers used for agricultural[2]. Concurrently, northern lakes (>45°) have been observed to be increasing in their dissolved organic matter (DOM) concentrations. Multiple factors, including land use, land cover, atmospheric acid deposition, and precipitation-driven runoff, are responsible [3]. It is still poorly understood how increased DOM and nutrient inputs will impact nutrient cycling and biological communities. Therefore, we must understand how various ecological and biogeochemical processes affect the Great Lakes. Microbial communities are of special interest since they are heavily involved in the nitrogen cycle by carrying out processes such as nitrogen fixation, nitrification, and denitrification. Microbes also play a crucial role in phosphate solubilization and organic matter decomposition, thereby making them essential for nutrient availability and cycling in aquatic environments. Microbial communities can fluctuate in their assemblages [4], [5], their metabolism [6], morphology [7], and stoichiometry [8], [9] as responses to environmental variables such as DOM and nutrients. It has been established that bacterial stoichiometry, metabolic capacity, and morphology change in response to particulate and dissolved C, N, and P in the environment these communities find themselves in, but these studies are often done *in vitro* and cannot account for natural conditions. Not only would disruptions caused by inputs of nutrients and DOM change assemblages and physiological traits of microbes, but they would also perturb biogeochemical cycles. Additionally, changes in stoichiometric ratios (C:N:P) can have impacts on essential fatty acid content and overall, the quality of food that lower trophic levels (bacteria and phytoplankton) could provide for higher trophic levels (zooplankton and fish). What we propose is an *in situ* reciprocal transplant experiment where microbial communities from Lake Huron and Lake Superior will be collected and exposed to the nutrient conditions of each lake, to uncover the inept ability of the microbial communities to adapt to changing nutrient conditions. In doing so, the proposed research will help inform management agencies of the present state of water quality in Lakes Huron and Superior while providing information on how increased nutrient and DOM loading into the Great Lakes may affect biological communities and important biogeochemical cycling.

***Goals and Hypothesis:*** The goal of my proposed research is to investigate microbial community adaptation to different nutrient and DOM concentrations. **Objective 1:** Characterize the response of microbial stoichiometry to changes in particulate and dissolved organic matter **Hypothesis 1 (H1):** Microbial communities from oligotrophic systems will be less flexible in their stoichiometry when compared to communities from eutrophic systems. **Objective 2:** Evaluate microbial community adaptation to altered nutrient concentrations and environmental conditions **Hypothesis 2 (H2):** Oligotrophic systems will have a lower functional redundancy when compared to eutrophic systems, marked by the presence of more rare taxa and inability to metabolize a variety of carbon sources.

***Relation to MISG strategic plan:*** The proposed research directly related to the 2024-2027 Michigan Sea Grant Strategic plan **Goal 1, Desired Outcome 1.2,** “Educators, students, and lifelong learners have current information and innovative tools that meet or exceed relevant standards and practices”. **Goal 3, Desired Outcome 3.2,** “Evidence-based science, traditional and local, and innovative solutions inform and improve management and conservation of coastal habitats”. **Goal 7, Desired Outcome 7.1,** “Scientific understanding, including traditional and local knowledge, provides foundational information, and all community members understand the impacts of changing conditions and coastal hazards and can prepare, respond and adapt”.

***Experimental Design:*** To investigate **H1 and H2,** bacterial communities from Lakes Superior and Huron will be collected, and be used for an *in situ* reciprocal transplant experiment using dialysis bags (14,000 kDa MWCO). Lake Superior and Huron communities will be collected, and the microbial community of one lake will be placed into dialysis bags and incubated in the other lake. Water samples will be analyzed for particulate C, N, and P of the bacterial communities and the seston inside the dialysis bag. Additionally, morphological traits will be determined via flow cytometry, changes in community assemblage will be characterized using 16S rRNA gene sequencing, and functional capacity will be measured by carbon substrate utilization using BIOLOG ecoplates. Translationally active microbes will be measured using biorthogonal amino acid tagging (BONCAT). To measure DOM and dissolved nutrients, 0.45 mm filtered water samples will be analyzed for dissolved organic carbon (DOC), total nitrogen (TN), nitrogen species (nitrate, nitrite, and ammonium), and soluble reactive phosphorus (SRP). Fluorescent dissolved organic matter will be measured by fluorescence excitation-emission matrix spectroscopy. Initial samples from each host lake will also be collected for analysis of nutrient content and microbial community, then sampled again following a 14-day incubation. CTD and light profiles for each lake will also be collected for each host lake at the beginning and end of each incubation. Community adaptation will be measured via plasticity in stoichiometry, morphology, translational activity, and community assemblage. Functional redundancy and metabolic capacity will be captured by using carbon substrate consumption microbial assemblages. A Bray-Curtis dissimilatory matrix will be used to compare bacterial communities to themselves at the start of the incubation, and to the host lake’s communities.

***Timeline:*** Sampling will be conducted in May of 2026. Data analysis will be done in the fall of 2026. In Spring 2028, a manuscript will be drafted for publication. The results of this study will be included as a chapter in my dissertation, and I will tentatively defend it in spring 2029.

***Products:*** The outlined experiments and their findings will be incorporated into my dissertation as a chapter and presented at the 2028 IAGLR meeting. Finally, a manuscript will be prepared and submitted to the *Journal of Great Lakes Research.*

***References:***

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