***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 misunderstood how increased DOM, and nutrient inputs will impact nutrient cycling and biological commmunities , therefore, we must understand how various ecological, 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 role in phosphate solubilization and organic matter decomposition, making them imperative 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 asDOM, and nutrient availability. It has been established that bacterial stoichiometry, metabolic capacity, and changes in relation to particulate and dissolved C, N, and P of the environment. Not only would disruptions caused by inputs of nutrients and DOM and cause perturbations in biogeochemical cycles, but changes in stoichiometric ratios (C:N:P) can have impacts on essential fatty acids and overall, the quality of food that lower trophic levels (bacteria and phytoplankton) could provide for higher trophic levels (zooplankton and fish). Winter is an integral component of annual limnological processes, and the better we understand its role, the better we will be able to inform future research and apply effective measures to manage the Great Lakes and maintain healthy ecosystems and communities.

***Goals and Hypothesis:*** The goal of my proposed research is to investigate how seasonality impacts microbial communities, with an emphasis on the transition from winter to spring. **Objective 1:** Characterize the response of microbial stoichiometry to changes in particulate organic matter in conjunction with seasonal changes in temperature **Hypothesis 1a (H1a):** Oligotrophic systems will be more susceptible to temperature effects on bacterial stoichiometry, shown by an increase in P content and cell size. **Hypothesis 1ba (H1b):** Less severe winters will show a moderate response in stoichiometric plasticity. **Objective 2:** evaluate microbial community resilience to altered nutrient concentrations and environmental conditions **Hypothesis 3 (H3):** oligotrophic systems will have a lower functional redundancy when compared to eutrophic systems, marked by more specialized taxa when compared to eutrophic systems making oligotrophic systems less resilient to changes in environmental conditions.

***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:* Experiment 1:** To investigate **H1 and H2,** bacterial communities from Lake Superior, Huron, and Erie will be collected, and an *in situ* reciprocal transplant experiment using dialysis bags (14,000 kDa MWCO, cellulose membrane, 76 mm flat width, to allow for substrates smaller than 12,000 kDa to diffuse across the membrane, allowing for exposure to ambient temperature and nutrient condition will be conducted) where Lake Superior and Huron communities will be swapped and repeated for Lake Huron and Lake Erie communities.The bacterial response to being transplanted into a different environment will be tracked throughout 48 hrs with sub-sampling taken at 12 h intervals (0, 12, 24, 36, and 48). Samples from each host lake will also be collected for analysis wat the beginning and end of the incubation period. 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, and changes in community assemblage will be characterized using 16S rRNA gene sequencing. Physical and chemical parameters such as ice cover (when applicable), dissolved oxygen, pH, and water temperature will also be measured. To measure DOM, 0.45 mm filtered water samples will be analyzed for dissolved organic carbon (DOC) and total nitrogen (TN) using a Shimadzu TOC-L, and fluorescent dissolved organic matter will be measured by fluorescent microscopy and excitation-emission matrices using a Horiba Aqualog. **H3** will be conducted similarly to **H1** and **H2**, but field work will not be conducted until the following winter (2027) to record annual variation in winter conditions. Winter severity will be measured in both experiments 1 and 2 and will be classified as ice depth (cm), as ice depth is closely linked to water temperature. Community resilience will be measured via plasticity in stoichiometry, morphology, and community assemblage. A Bray-Curtis dissimilatory matrix will be used to compare bacterial communities to themselves throughout the incubation, and to the host lake communities.

***Timeline:*** Sampling for experiment 1 will be done in January and May of 2026. Data analysis for experiment 1 will be done in the fall of 2026. Field work for experiment 2 will be conducted during January and May of 2027, with data analysis being conducted in the fall of 2027. In Spring 2028, a manuscript will be drafted. I will tentatively defend my dissertation 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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