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Assessment of nitrogen fixation rates in the Laurentian Great Lakes

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ABSTRACT

Nitrogen fixation (N_{Fix}) is an important, yet understudied, microbial process in aquatic ecosystems, especially in the Laurentian Great Lakes (LGL). To date, a dearth of nitrogen fixation rate measurements exists in the LGL, are from temporally isolated studies, and were collected primarily from near-shore and surface water environments. Evidence of nitrogen accumulation across the Laurentian Great Lakes suggest that we do not have a firm grasp on nitrogen cycling in large lakes. Thus, we sought to quantify the spatial variability of N_{Fix} in the LGL. We found lakes are significantly different in N_{Fix} rates from one another and that rates are depth dependent. Overall mean surface N_{Fix} rates of Lakes Superior, Michigan, Huron, Erie and Ontario were 0.024, 0.020, 0.069, 0.145, and 0.078 (nmol N_2/L/hr), respectively. Likewise, we found the Western, Central and Eastern basins of Lake Erie are significantly different in N_{Fix} rates (0.1540, 0.1032, 0.0738 nmol N_2/L/hr). However, we found no significant difference in N_{Fix} rates between near and offshore sites in Lake Erie, which may have been biased due to a cyanobacterial bloom containing a nitrogen-fixing *Dolichospermum sp.* Linear regression models indicate N_{Fix} is generally positively correlated with chlorophyll-*a* concentration and negatively correlated with oxidized nitrogen species concentrations. However, Lakes Erie and Huron exhibited a positive linear relationship with oxidized nitrogen, suggesting that N_{Fix} may persist to meet cellular and community nitrogen demands. Together, our data highlight N_{Fix} is important despite the presence of abundant nitrogen in all LGL.

Introduction

Nitrogen fixation (N_{Fix}), or diazotrophy, is an important, microbially mediated process that converts dinitrogen (N_2) gas to ammonia (NH_3), and is found in diverse lineages of symbiotic, associative, and free-living bacteria (Dixon and Kahn, 2004). In aquatic systems, N_{Fix} rates are typically correlated with light availability (Paerl, 1990), suggesting phototrophic organisms like cyanobacteria, are primarily responsible for N_{Fix}. However, work in marine environments indicate that cryptic nitrogen fixing microorganisms other than cyanobacteria are important to the nitrogen cycle (Zehr et al., 1998; Delmont et al., 2018). This would suggest that in most aquatic systems N_{Fix} is an emergent community level property governed by diverse organisms. At the ecosystem level, factors like nutrient stoichiometry (nitrogen:phosphorus), grazing, micronutrients, turbulence, among others (Howarth et al., 1988; Vitousek et al., 2002; Schindler et al., 2008; Higgins et al., 2018) all influence N_{Fix} rates. Nonetheless, N_{Fix} is a linchpin in the nitrogen cycle that re-mobilizes nitrogen lost to denitrification processes (Small et al., 2016) and eases nitrogen demand in both freshwater and marine environments (Shanmugam et al., 1978; Howarth et al., 1988; Capone and Montoya, 2001; Capone et al., 2005). However, N_{Fix} is energetically expensive, oxygen sensitive, and typically inhibited by excess dissolved inorganic nitrogen (DIN) (Gallon, 1992; Li et al., 2003; Beversdorf et al., 2013; Paerl, 2017). While diazotrophic organisms have evolved mechanisms to overcome oxygen sensitivity (Gallon, 1992; Church et al., 2005), nutrient sensitivity remains an enigma for N_{Fix}. Typically, N_{Fix} is thought to decrease with increasing concentrations of DIN (Howarth et al., 1988). Yet, nitrogen fixing cyanobacteria, with heterocysts, are often identified in waters replete with nitrogen (Chaffin et al., 2020; Sterner et al., 2020). Furthermore, in oligotrophic waters of the northern Atlantic Ocean, N_{Fix} rates are consistently higher than what would be expected based on total DIN (Landolfi et al., 2015). Together, this would suggest our understanding of the controls and constraints of N_{Fix} is lacking, and that long held dogmas may not adhere to all systems.

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Nitrogen has not typically been considered a limiting element in most freshwater systems (Schindler, 1977). However, it is evident that biological nitrogen limitation can occur under periods of high productivity (Montoya et al., 2002). Furthermore, nitrogen limitation due to denitrification promoted by anoxia and warm temperatures also suggest a correlation between nitrogen availability and nitrogen fixing microorganisms (Hecky, 1993; Downing et al., 1999; Bootsma and Hecky, 2003). In smaller lakes, autochthonous N_{Fix} and terrestrial nitrogen inputs are necessary to offset N_2 loss from sediment-associated denitrification and annamox processes (Loeks-Johnson and Cotner, 2020). Typically N_{Fix} is underappreciated in aquatic systems, yet N_{Fix} can comprise up to 85% of assimilated nitrogen by the phytoplankton community, thus helping offset biological nitrogen deficits (Salk et al., 2018). Long-term studies in Lake 227 (Experimental Lakes Area) highlight that N_{Fix} has accounted for 69–86% of total nitrogen loading in the epilimnion at the onset of bloom development and 72–86% of total nitrogen loading May–October, commonly associated with bloom season (Higgins et al. 2018). Together, this would suggest that in most lake systems, N_{Fix} is a necessary process whose contribution to whole lake nitrogen budgets is undervalued especially when considering the volume of large lakes. Because of the size of large lakes, offshore chemical and physical environments may be substantially different from the nearshore environment (Mugidde et al.,2003; Kovalenko et al., 2019). The resulting ecological gradients likely have broad implications to nutrient cycles, like the nitrogen cycle.

N_{Fix} in the Laurentian Great Lakes (LGL), like many aquatic systems (Vitousek et al., 2002), is understudied and potentially undervalued. Initial work in the upper LGL quantified N_{Fix} rates in concentrated biomass fractions > 64 μm but did not concentrate biomass < 64 μm to measure N_{Fix} (Mague and Burris, 1973). Microorganisms, including colonial cyanobacteria, would easily pass through this course filtration. Thus, the results from this study indicated that nitrogen fixation rates in the upper LGL, specifically Lake Superior were insignificant to nonexistent. However, we know that many diazotrophs are small, typically slow growing (Agawin et al., 2007) and thus are rare members of the microbial community (Delmont et al., 2018). Thus, Mague and Burris (1973), likely underestimate N_{Fix} by not incorporating free-living (microbes caught on a 0.22 μm filter) or particle-associated (microbes caught on a 3.0 μm filter) microorganisms. Additionally, these measurements focused on nearshore surface waters (1–2 m), which likely missed key N_{Fix} populations that reside deeper in the water column (MacGregor et al., 2001). In Lake Michigan, putative N_{Fix} populations are transcriptionally active (e.g., detection of *nifH* genes in bulk mRNA extracts) and consisted primarily of Cyanobacteria (heterocyst expressing) and Alpha-, Beta- and Gammaproteobacteria (MacGregor et al., 2001). Additionally, MacGregor et al. (2001) found isotopically depleted organic nitrogen pools in the water column, suggesting N_{Fix} is supplying isotopically light nitrogen to the community. In Lake Superior an accumulation of nitrate has created a stoichiometric imbalance between nitrogen and phosphorus (Sterner et al. 2007). Interestingly, characterization of the isotopic signatures of the oxygen and nitrogen in the nitrate show both are vastly different from depositional (rainwater) and riverine nitrate pools, suggesting that nitrate increase is through autochthonous processes (Finlay et al., 2007). Taken together, there is much to understand about N_{Fix} in the LGL and what factors govern rates. To this end, we sought to quantify the extent and rates of diazotrophy across the LGL. We hypothesized that N_{Fix} will exhibit spatial heterogeneity (both with water column depth, between stations and between lakes) and that nitrogen availability and demand in the lakes will influence rates.

Materials and methods

Sampling locations and descriptions

Samples from Lakes Michigan, Huron, Erie, and Ontario were collected aboard the *R/V Lake Guardian* in late July and early August 2019, and samples from Lake Superior were collected aboard the *R/V Blue Heron* in early August 2019. Sampling stations for the Lakes Michigan, Huron, Erie and Ontario were chosen based on the master stations currently sampled by the Environmental Protection Agency (EPA) Great Lakes National Program Office's (GLNPO). Lake Superior stations were based on an opportune cruise and do not coincide with traditional EPA-GLNPO stations. Due to weather, we faced sampling time constraints and were unable to quantify nutrients or chlorophyll-*a* concentrations in Lake Superior. Stations included in our sampling varied by total water column depth and proximity to shore (Fig. 1). Given the vast difference of physical characteristics of each lake and within each lake, sampling depths are as follows; an epilimnion surface (2 m) sample (constant at all stations), mid-water column hypolimnion based on max station depth, and hypolimnion near-bottom water (~1 m from bottom) (Electronic Supplementary Material (ESM) Table S1). For the western basin of Lake Erie, only a surface and near-bottom water sample were collected due to its shallow depths. The mid-water collection did not coincide with the thermocline, which was not present at all stations.

Water sampling and quantification of nitrogen fixation

Water samples taken aboard the R/V *Lake Guardian* were collected from discrete depths (Table 1) using a CTD-rosette water sampler equipped with twelve – 8 L Niskin bottles and outfitted with a Sea-Bird multi-parameter profiler (conductivity, temperature, and depth). A similar system is used by the R/V *Blue Heron*. Two liters of water were collected in triplicate from each depth (8 L total) and concentrated onto a 0.22 μm filters (MF- Millipore Membrane filter, 2 L per filter) using a vacuum manifold at low pressures to minimize cell breakage. In preliminary testing in Lake Superior, we determined that approximately 2 L of water was necessary to capture enough biomass to reliably quantify rates in oligotrophic waters. We recognize that ultrasmall nitrogen-fixing microbial populations may pass through the 0.22 μm filter and that further study is necessary to ascertain if they occur and are active in freshwater systems. In areas of high biomass, such as Lake Erie, water was filtered until the filter was clogged, which were all below 1 L. Filtrate volume was noted, and final nitrogen fixation values were corrected based on volume filtered. A caveat to this approach is that we may under or over-estimate N_{Fix} depending on whether the dominant nitrogen fixing microorganism is associated with larger particles or free-living.

Nitrogen fixation rates were quantified using an adapted acetylene reduction assay, ARA (Stewart et al., 1967). The ARA, rather than stable isotope method, was chosen due to its straightforward field set up, ability to compare rates to older studies, and the ability to quantify acetylene and ethylene in-house with an existing gas chromatograph. Using methods outlined by Capone and Montoya (2001), following filtration, filters were transferred to 50 mL serum vials, submerged in 25 mL of filtrate (lake water) from the same depth it originated from, sealed with a chlorobutyl rubber stopper (Wheaton), and sealed with an aluminum cap. Acetylene gas was generated shipboard prior to sampling by combining 10 g calcium carbide (CaC_2) and 100 mL of deionized water in a 250 mL side arm flask attached to a 1 L Tedlar gas sampling bag (EnviroSupply & Service). Samples were spiked with 1 mL of acetylene gas giving

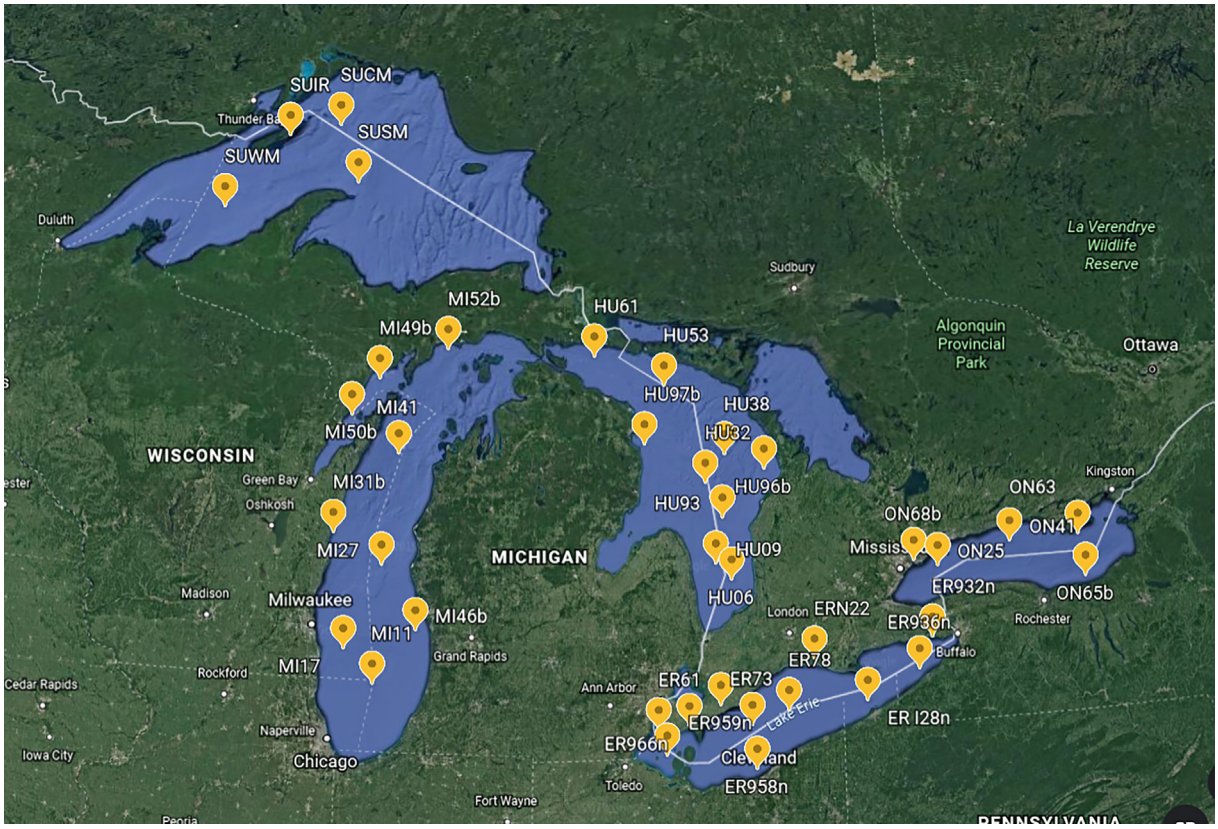

Fig. 1. Location of sampling stations chosen for our nitrogen fixation study.

Table 1
Mean whole lake N_{fix} rates (nmol N_2/L/hr^{-1}) observed and the pairwise statistical comparisons of within lake and between lake depths.

	Superior			Huron			Michigan			Erie			Ontario		
N_{Fix} Rate	mean	stdev	n	mean	stdev	n	mean	stdev	n	mean	stdev	n	mean	stdev	n
Whole Lake	0.015	0.020	36	0.043	0.079	72	0.01	0.009	81	0.099	0.126	99	0.039	0.053	45
Surface	0.024	0.034	12	0.069	0.134	24	0.020	0.012	27	0.145	0.089	33	0.078	0.079	15
Mid	0.013	0.004	12	0.035	0.012	24	0.012	0.007	27	0.080	0.035	33	0.023	0.005	15
Bottom	0.005	0.003	12	0.026	0.010	24	0.008	0.002	27	0.069	0.027	33	0.016	0.003	15
Statistical Comparisons between depths (p-value, α =0.05)															
Surface v. Mid			0.71			0.054			**0.001**			0.095			**<0.0001**
Surface v. Bottom			**0.042**			**0.003**			**<0.0001**			**0.003**			**<0.0001**
Mid vs Bottom			**0.012**			**0.027**			**0.003**			0.21			**0.002**

an initial acetylene headspace concentration of approximately 0.15 atm, which is near the minimum recommended by Capone and Montoya (2001). Samples were incubated at near *in situ* conditions (temperature and light) aboard the ship for 24 h. For deep samples, incubations were done in the dark and with an appropriate temperature regime. After 24 h, the incubations were terminated using 5 mL of trichloroacetic acid (TCA) and stored in the dark at 4 °C until measurements could be made in the lab. Due to a lack of N_{Fix} studies on the Great Lakes, we choose to incubate samples for 24 h based on preliminary work in Lake Superior, knowing that would be our high-end for incubation time.

Acetylene and ethylene gas concentrations (peak area integration) were measured using an Agilent 6890 Plus GC System equipped with a flame ionization detector (FID) and a GS-Carbon Plot column 100/120 mesh (Agilent 113–3122). The GC-FID parameters are as follows: the carrier gas helium was set at a flow rate of 30 cm/s, the oven temperature was held isothermally at 125 °C for 3 min, with a split injection of 20:1 (carrier gas to sample) at 250 °C. Sample injection volumes were 100 μL. The retention times for acetylene and ethylene were observed at 1.8 and 1.9 min, respectively. For each station and at each depth, the following series of blanks and controls were used: (1) kill standard (Filtered biomass, filtered water and TCA immediately added); (2) acetylene + sterile DI water; and (3) acetylene + filtrate. The kill standard is necessary to show the performance of TCA at terminating a sample. The acetylene + DI water standard determined the purity of the acetylene used to incubate each sample, and the acetylene + filtrate was used to account for other sources or N_{Fix} occurring in cell fractions that can pass through a 0.22 μm filter. Likewise, a standard containing both equal concentrations of acetylene and ethylene was used to determine the effectiveness of the column to separate the gases. Calculations of nitrogen fixation rates (nmol N_2 /L/hr) were based on methods outlined in Capone and Montoya (2001), which account for the partitioning of the acetylene and ethylene gasses between the headspace and dissolved in the medium. We used a correction ratio of 4:1 (ethylene gas to N_2) instead of a traditional 3:1, to account for the reduced H_2 evolution (hydrolysis) associated with the reduction of

acetylene (Capone, 1993). Latitude and Longitude of stations and nitrogen fixation rate data is available from PANGAEA, https://doi.pangaea.de/https://doi.org/10.1594/PANGAEA.920001.

Statistical analysis and graphing

R Studio (1.1463) using R base version 3.5.2 (R Studio Team, 2016) was used to perform all statistical analyses. Data normalcy was tested using the Shaprio-Wilk's test in the dplyr r-package (Wickham et al., 2018). Due to unequal sample sizes (between lakes) and non-normal data, we chose the nonparametric Wilcoxon Test and the Dunn-Kruskal-Wallis (FSA package) (Ogle et al., 2020) with a Holm's p-value adjustment to test for pairwise multiple comparisons of N_{Fix} rates. Linkages between N_{Fix} rates and environmental variables (*i.e.,* total nitrogen, total phosphorus in filtrate, total phosphorus in bulk water, chlorophyll-a, alkalinity, and total oxidized nitrogen) were investigated using linear regression modeling, lmSupport package (Curtin, 2018), significant models were based on AIC scores and QQ plots. Correlations were calculated using the Kendall method, car package (Fox and Weisberg, 2011). Environmental chemistry data associated with the cruise were downloaded from the USEPA Great Lakes Environmental Database (GLENDA). All graphs were created using with ggplot2 (Wickham, 2016). Google maps and ggmap (Kahle and Wickham, 2013) were used to construct maps necessary for visualizing sample locations and nitrogen fixation analyses.

Results

Overall, Lake Erie had the highest N_{fix} rates relative to the other LGL, while Lake Superior had the lowest rates measured (Fig. 2 and Table 1). Interestingly, N_{fix} rates in Lake Ontario's surface waters were nearly as high as Erie, but its mid and deep depths were substantially lower. Pairwise comparisons of N_{fix} rates show that each lake is statistically significant from one another except for Huron *vs.* Ontario and Michigan *vs.* Superior (Table 2). Overall, water column depth was a significant predictor of N_{fix} rates for surface *vs.* middle (p-value = 0.002) and surface *vs.* bottom (p-value = <0.001) but not for middle *vs.* bottom (p-value = 0.2). However, each lake responded differently to the depth gradient (Fig. 2). The shallowest of the LGL (by mean depth), lakes Erie and Huron, showed little difference between surface, middle and bottom waters, while lakes Ontario and Michigan showed large differences with depth. For Superior, differences were consistently observed between surface and deep-water samples. Nonetheless, consistently we observed significant differences between surface and deep-water samples. Within lakes we observed large variability between stations, especially in Lake Erie's western basin and western Lake Ontario (Fig. 3). Given the distinctiveness of Lake Erie's basins, we compared N_{fix} rates in each basin and throughout the water column. While water depth was not significantly correlated with N_{fix} rates, fixation rates were significantly different between basins (Western, Central and Eastern), such that the western basin N_{fix} rates (0.154 nmol N_2/L/hr) were nearly twice as high as the

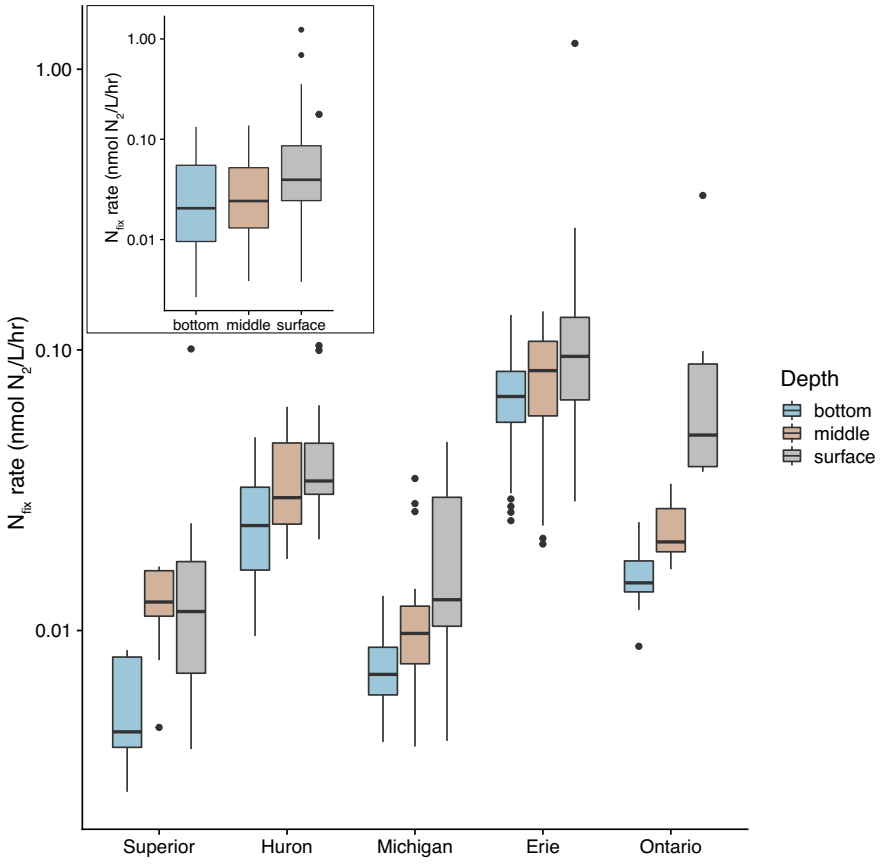

Fig. 2. Box plots of N_{fix} rates of LGL with respect to the depth at which samples were taken from. Inset graph shows the overall patterns of nitrogen fixation for the entire LGL study.

Table 2
Pairwise statistical comparisons of whole lake N_{fix} rates. All values represent the P-value of the comparison.

	Superior	Huron	Michigan	Erie	Ontario
Superior	–				
Huron	**<0.0001**	–			
Michigan	0.97	**<0.0001**	–		
Erie	**<0.0001**	**<0.0001**	**<0.0001**	–	
Ontario	**<0.001**	0.77	**<0.0001**	**<0.0001**	–

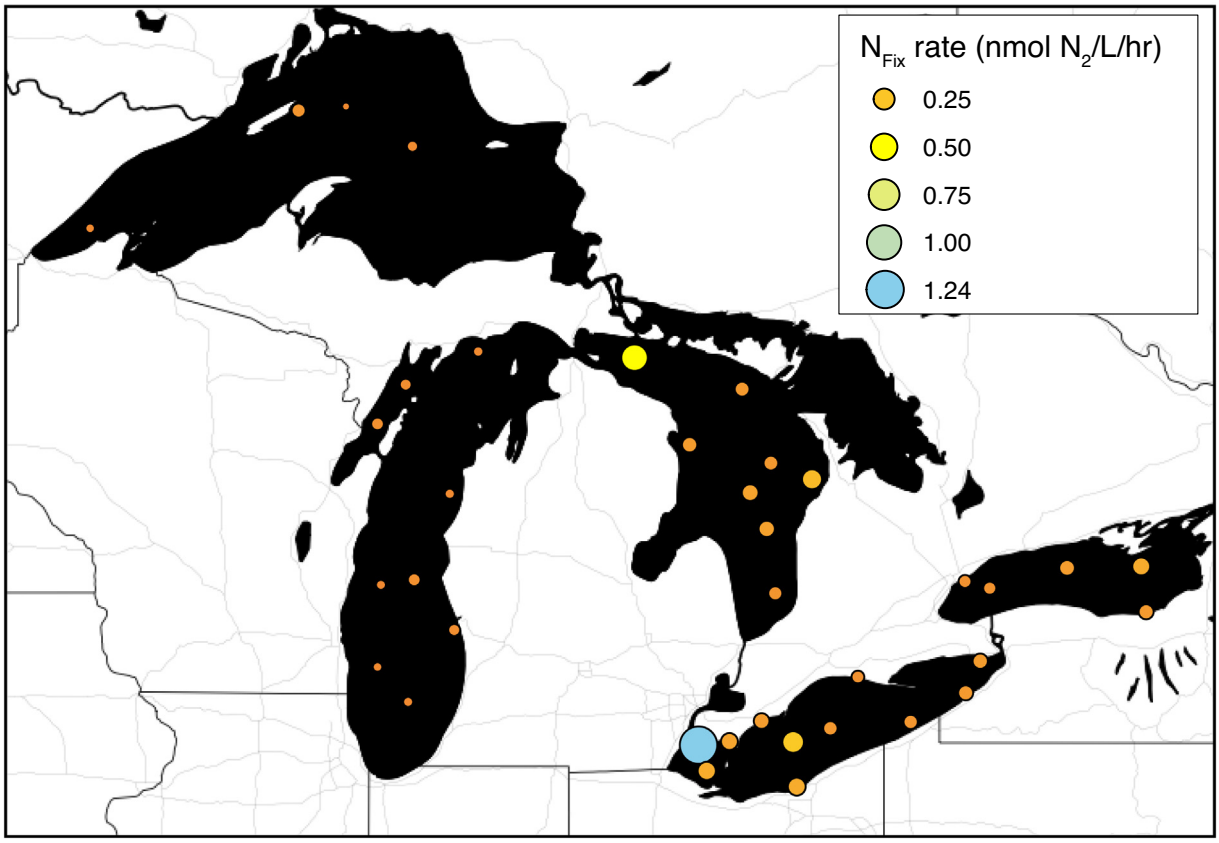

Fig. 3. Surface N_{fix} rates (nmol N_2/L/hr) at each sampling station across the LGL. For values of high and low nitrogen fixation see Fig. 2.

central (0.103 nmol N_2/L/ hr) and the eastern basin (0.074 nmol N_2/ L/hr).

Linear regression models identified oxidized nitrogen and chlorophyll-*a* concentrations as significantly correlated with N_{Fix} rates (see ESM Fig. S1 and Fig. 4A&B, p-values < 0.001 and 0.017, respectively, R^2 values = 0.16 and 0.38). These data were not available for Lake Superior. Further examination using Kendall rank correlations also found oxidized nitrogen and chlorophyll-*a* as predictors of N_{Fix} rates (P-values, <0.001 and < 0.001, Tau values, −0.349 and 0.453, respectively), suggesting a possible predictive capability for these parameters. A multiple linear regression model, combining total oxidized nitrogen and chlorophyll-*a*, was also significant (P-value = 0.038, R^2 value = 0.27), and the interactions of chlorophyll-*a* and oxidized nitrogen were not significant in the model.

Discussion

In all aquatic systems, N_{Fix} is performed by photosynthetic and non-photosynthetic diazotrophs (Zehr et al., 1998), and their contribution will likely change as a function of light availability. Overall, we found significant differences with depth, especially in surface *vs.* bottom samples (Fig. 2 and Table 1). This would suggest that in the upper water column, phytoplankton are important diazotrophs in the summer months across the LGL. In Lakes Malawi, Tanganyika and Victoria, three eutrophic large lakes, N_{Fix} is also correlated with light concentrations, depth and season (Mugidde et al., 2003; Gondwe et al., 2008). Furthermore, N_{Fix} microorganisms have been found to comprise a significant proportion of both phytoplankton and periphyton communities during bloom season and contribute a significant portion of annual nitrogen inputs to the African Great Lakes (Bootsma and Hecky, 2003; Mugidde et al., 2003). While these studies show N_{Fix} was greatest in the photic zone, depth profiles across these large lakes indicate that non-photosynthetic diazotrophs are also important, as N_{Fix} continues to occur in deep, aphotic waters. In Lake Michigan, several non-photosynthetic diazotrophs were actively transcribing the *nifH* gene (MacGregor et al., 2001). Likewise, heterotrophs accounted for ~70% of *nifH* reads in surface water metagenomes in Sandusky Bay, Lake Erie (Davis et al., 2015). These studies highlight that non-photosynthetic diazotrophs are present and likely actively fixing nitrogen throughout the water column. Similarly, in Lake Taihu, sequencing and quantification of the *nif*H gene suggests diverse

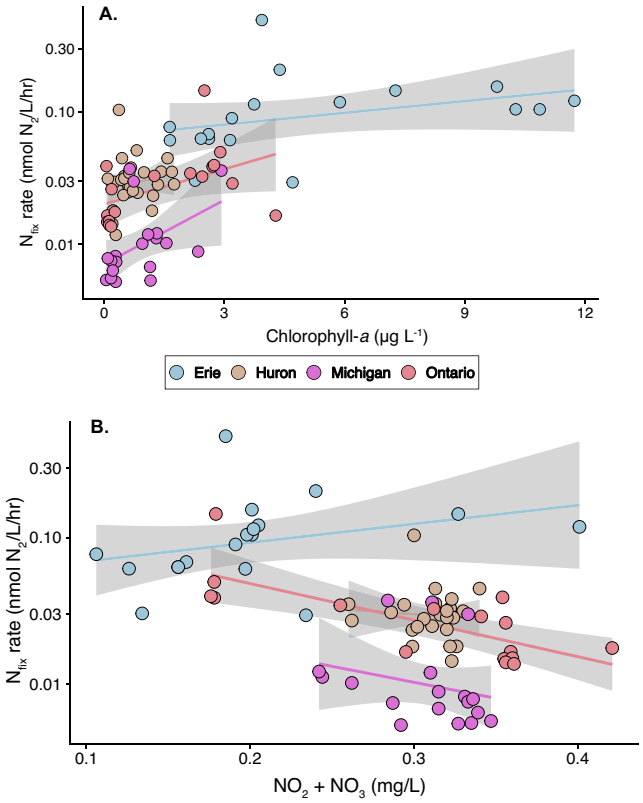

Fig. 4. Environmental factors that were significantly correlated with N_{FIX} rates. (A) Chlorophyll-*a* trend observed for each of the LGL separately. (C) Oxidized nitrogen trend observed for each of the LGL separately. For overall patterns see ESM Fig. S2. Data are plotted in log–log scale for visualization.

diazotroph communities reside in sediments and contribute to the lake wide nitrogen budget (Tian et al., 2021). In our Lake Erie N_{fix} measurements, we did not see significant depth dependence (surface *vs* bottom waters, Fig. 2). These results are likely due to the shallow nature of Lake Erie, but also highlight that deep water diazotrophy with rates similar to surface waters may be an underappreciated source of reduced nitrogen in Lake Erie. It should be noted that we sampled Lake Erie during a peak biomass growth time and that rates will likely be quite variable and different from early season/late season rates. Furthermore, in large, deep temperate lakes that have relatively stable temperatures for much of the year, diazotrophy may not follow seasonal trends seen in the epilimnion. For example, the majority of Lake Superior's water column is less than 5 °C (See ESM Fig. S2). Rather, these microorganisms may be capable of N_{Fix} at rates similar to what were measured in our study throughout the year, and thereby could be a significant source of reduced nitrogen throughout the year. However, seasonal studies are necessary to validate this. Taken together, studies quantifying nitrogen budgets that account for only surface water nitrogen fixation may be grossly underestimating by omitting whole water column and sediment rates.

The LGL represent a hydrologic continuum, however, stark differences in nutrient conditions exist between the lakes (Sterner, 2021). Likewise, we found N_{fix} rates and patterns differed among the lakes and differentially responded to oxidized nitrogen concentrations (Fig. 4B). Previous work has shown that N_{fix} is controlled by many factors (e.g., light, temperature, micronutrients; Paerl, 1990), but nitrogen availability is particularly important for cyanobacterial nitrogen fixers (Li et al., 2003, Hampel et al., 2019). Thus, we expected that N_{fix} rates would decrease with increasing oxidized nitrogen concentrations. However, Lake Erie, and to some extent Lake Huron, did not follow this pattern and displayed stable rates despite ample oxidized nitrogen (Fig. 4B). Paerl and Otten (2016) have noted there is conflicting evidence for DIN influence on N_{fix} rates and abundance of diazotrophic cyanobacteria. In our study, the observed trend may speak to the cellular nitrogen preference (e.g., uptake of reduced *vs.* oxidized) in Lake Erie. Interestingly, in Lake Erie the chlorophyll-*a* trend also follows a similar trend to oxidized nitrogen, which is contrary to the other lakes that increase rates with concomitant increase of chlorophyll-*a*. Previous studies have suggested that N_{Fix} activity still occurs regardless of DIN concentration, due to cellular nitrogen demands of the microbial community (Moisander et al., 2012; Chaffin et al., 2013; Harke and Gobler, 2015). Thus, the community structure of the water column, both prokaryotic and eukaryotic, may have direct influence on the rates of N_{Fix}. Coincidentally, we collected samples from a Lake Erie *Microcystis* bloom at station ER61 (a western basin, offshore station on August 13th, 2019, Fig. 1). In our sampling, we observed *Dolichospermum* sp. with visible heterocyst structures interspersed in the bloom sample. At ER61 nitrogen fixation rates were ~ 11x higher than the mean rate of Lake Erie and ~ 9x higher than stations in the Western Basin. Identification of multispecies cyanobacterial blooms, whereby N_{fix} cyanobacteria are interspersed with primary bloom species, are commonplace (Chaffin et al., 2013, 2019; Salk et al., 2018). Likewise, in ELA Lake 227 observed shifts from a non-diazotrophic community (~80% of total biomass) to a diazotroph dominated community (~95% of the total biomass associated with *Aphanizomenon sp.*) suggest the importance of diazotrophic cyanobacteria to maintaining phytoplankton biomass (Higgins et al., 2018). Although evident in previous studies, more work is needed to understand the interspecies interactions between diazotrophic cyanobacteria and other cyanobacteria during bloom transitions (Paerl and Otten, 2016; Chaffin et al., 2019). Furthermore, future work understanding the roles of non-photosynthetic N_{fix} in bloom formation and maintenance is necessary, as these organisms may have close association with toxin producing cyanobacteria (Jankowiak and Gobler, 2020; Smith et al., 2021).

Our work suggests, there may be no one size fits all mechanism underpinning N_{fix} across the LGL and that each lake must be treated individually. However, we point out that our study did not measure reduced inorganic nitrogen species, which may be a stronger regulator of N_{Fix} rates (Flores and Herrero, 2005). The LGL have undergone significant changes last century, as anthropogenic climate change has driven mean lake temperatures and ice dynamics, while land use changes and invasive species have altered the chemistry of the LGL (Mao and Cherkauer, 2009; Hayhoe et al., 2010). Lake Superior, while thought of as the most pristine of the LGL, is rapidly changing thermally (O'Beirne et al., 2017; Sterner, 2021) and chemically. Nitrogen accumulation is occurring throughout the LGL (Sterner, 2021), and Lake Superior has seen an accumulation of nitrate over the last century (Sterner et al., 2007; Finlay et al., 2007). Given the large N:P stoichiometric imbalance, one would predict that N_{fix} should be nonexistent. Yet we quantified rates that are on par with those of Lake Michigan and other oligotrophic lakes (Howarth et al., 1988). Thus, the general assumption that N_{fix} activities cease unless under N-limitation are not consistent and suggest that diazotrophic microorganisms in systems like Lake Superior are not metabolically hampered by external nitrate or nitrite concentrations. Rather, colimitation of other elements, such as iron or phosphorus, may throttle N_{Fix}. Furthermore, N_{fix} in stoichiometrically imbalanced systems is not limited to freshwater systems, as the northern Atlantic Ocean sees similar inconsistences in N_{fix} rates (Landolfi et al., 2015). Together this suggests that the contribution of N_{fix} in aquatic systems may be overlooked based on presumptions.

As the LGL continue to evolve in response to anthropogenic stressors driven largely by climate change, it is necessary to holistically understand how current biogeochemical cycles will respond. Here we show that N_{fix} should not be an overlooked microbial process and warrants further study across the LGL. While our study offers only a snapshot in time, we highlight that N_{fix} does not necessarily adhere to traditional presumptions, and blanket predictors of N_{fix} may not be suitable for predicting N_{Fix} in the LGL. Furthermore, temporal changes in N_{fix} are likely, as lake temperatures increase and biological demands for nitrogen increase due to longer growing seasons (Adrian et al., 2009). Finally, it is apparent that a comprehensive study of N_{Fix} is necessary to understand the temporal variability across the LGL to better inform ecological models.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jglr.2021.07.005.

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