

Spatial and temporal variation in nitrogen fixation and its importance to phytoplankton in phosphorus-rich lakes

Nicole M. Hayes¹  | Alain Patoine² | Heather A. Haig¹ | Gavin L. Simpson³  |
Vanessa J. Swarbrick^{1,4}  | Emma Wiik^{3,5} | Peter R. Leavitt^{1,3,6} 

¹Limnology Laboratory, Department of Biology, University of Regina, Regina, Saskatchewan, Canada

²Université de Moncton, Shippagan, New Brunswick, Canada

³Institute of Environmental Change and Society, University of Regina, Regina, Saskatchewan, Canada

⁴Alberta Environment and Parks, Edmonton, Alberta, Canada

⁵Marine Centre Wales, School of Ocean Sciences, Bangor University, Menai Bridge, Anglesey, UK

⁶Institute of Global Food Security, Queen's University Belfast, Belfast, UK

Correspondence

Nicole M. Hayes, Department of Ecology, Evolution, and Behavior, University of Minnesota, St. Paul, MN, U.S.A.
Email: hayes.nicolemarie@gmail.com

Funding information

Canada Foundation for Innovation; Canada Research Chairs; Queen's University Belfast

Abstract

1. Limnological theory posits that phosphorus (P) limits primary production in freshwater lakes, in part because fixation of atmospheric nitrogen (N_2) can compensate for limitations in nitrogen (N) supply to phytoplankton. However, quantitative estimates of the degree to which N_2 fixation satisfies planktonic N demand are rare.
2. Here we used biweekly sampling during summer in seven lakes over 2 decades to estimate both planktonic N_2 fixation and phytoplankton N demand. We further assessed the ability of biologically fixed N to satisfy N needs of primary producers in productive hardwater lakes.
3. Phytoplankton N requirements, derived from estimates of phytoplankton productivity and N content, were moderately synchronous ($S = 0.41$) among lakes (ca. $0.1\text{--}9.2\text{ mg N m}^{-3}\text{ hr}^{-1}$). In contrast, rates of N_2 fixation determined using isotopic natural abundance method (NAM; $0.002\text{--}3.2\text{ mg N m}^{-3}\text{ hr}^{-1}$), or heterocyte-based calculations ($0.10\text{--}1.78\text{ mg N m}^{-3}\text{ hr}^{-1}$), varied asynchronously ($S_{\text{NAM}} = -0.03$ and $S_{\text{Heterocyte}} = -0.11$) among basins, accounted for a median of 3.5% (mean $11.3\% \pm 21.6$) of phytoplankton demand, and were correlated to the abundance of Nostocales cyanobacteria when analysed using generalised additive models.
4. Overall, the total mass of fixed N accounted for a median of only 3.0% of the spring standing stock of total dissolved N in study lakes (mean $7.5 \pm 12.1\%$), with higher relative importance of fixed N in highly productive downstream lakes. Thus, while fixed N helps sustain primary productivity, particularly in years with high rates of N_2 -fixation, it does not appear to eliminate N limitation of phytoplankton growth in these P-rich hardwater lakes.

KEYWORDS

$\delta^{15}\text{N}$, cyanobacteria, nitrogen demand, nutrient limitation, stable isotopes

1 | INTRODUCTION

Crop agriculture, intensive livestock production, urbanisation and fossil fuel production have combined to triple the amount of

phosphorus (P) released from terrestrial ecosystems (Bennett, Carpenter, & Caraco, 2001; Carpenter et al., 1998) and the amount of biologically-available nitrogen (N) entering the biosphere (Bodirsky et al., 2014; Fowler et al., 2013; Vitousek, Menge, Reed,

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2018 The Authors. *Freshwater Biology* Published by John Wiley & Sons Ltd.

& Cleveland, 2013). Export of N and P to aquatic ecosystems induces cultural eutrophication, with increased primary production (Schindler, 1977), blooms of potentially-toxic phytoplankton (Smith, 1983), altered cycling of most macronutrients (Bennett et al., 2001; Glibert, Maranger, Sobota, & Bouwman, 2014), changes in food-web structure (Carpenter et al., 2001), and enhanced atmospheric exchange of greenhouse gases (Pacheco, Roland, & Downing, 2014; Schindler, Carpenter, Cole, Kitchell, & Pace, 1997). Current management strategies have not controlled eutrophication in all ecosystems (McCrackin, Jones, Jones, & Moreno-Mateos, 2016), possibly reflecting uncertainty over the need to regulate P supply alone (Paterson, Schindler, Hecky, Findlay, & Rondeau, 2011; Schindler, Carpenter, Chapra, Hecky, & Orihel, 2016; Schindler et al., 2008) or both N and P (Leavitt, Brock, Ebel, & Patoine, 2006; Leavitt et al., 2009; Paerl & Scott, 2010; Paerl et al., 2016).

To date, management strategies for eutrophied lakes have focused on reduction of P influx for multiple reasons (Carpenter et al., 1998; Schindler et al., 2008, 2016). First, algal abundance is correlated best to P concentration (Dillon & Rigler, 1974). Second, chlorophyll-*a* (Chl-*a*) concentrations increase *ca.* 10-fold following fertilisation of oligotrophic lakes with P + N + carbon (C) but not N + C (Schindler, 1977). Third, water quality often improves following diversion or treatment of P from wastewaters (Jeppesen et al., 2005; Schindler, 2006). Fourth, diversion of N from fertilised P-limited boreal lakes has little effect on primary production, possibly because of compensatory N₂ fixation by cyanobacteria (Paterson et al., 2011; Schindler et al., 2008, 2016). However, while these insights are highly relevant to boreal ecosystems, they may not be equally applicable in lakes in regions with P-rich geological substrates (Klassen, 1989; Leavitt et al., 2006) or where centuries of agricultural fertilisation have saturated soils with P (Bennett et al., 2001; Carpenter, 2005), increased P export to surface waters (Bennett et al., 2001; Carpenter et al., 1998), and created conditions in which N pollution can degrade water quality (Bunting, Leavitt, Gibson, McGee, & Hall, 2007; Dolman et al., 2012; Kilinc & Moss, 2002; Leavitt et al., 2006; Moss, Jeppesen, Søndergaard, Lauridsen, & Liu, 2013). Where N limitation is severe, more direct evidence is needed to demonstrate that biological fixation of N₂ by cyanobacteria (Beversdorf, Miller, & McMahon, 2013; Patoine, Graham, & Leavitt, 2006) is sufficient to offset N demand by phytoplankton assemblages (Findlay, Hecky, Hendzel, Stainton, & Regehr, 1994; Schindler et al., 2008).

To date, select whole-lake experiments have been instrumental in demonstrating that biological N₂ fixation can sustain lake eutrophication arising from moderate fertilisation with P alone (Higgins et al., 2017; Schindler et al., 2008). However, several lines of evidence suggest that N₂ fixation may be insufficient to meet N requirements of natural phytoplankton assemblages in some P-rich lakes. For example, mass-balance studies comparing biological N₂ fixation with other N sources suggest that planktonic cyanobacteria may supply only a small fraction (5–10%) of summer N influx to lakes in agricultural basins (Ferber, Levine, Lini, & Livingston, 2004; Leavitt et al., 2006; Patoine et al., 2006 but see Scott & Grantz, 2013). Second, soluble reactive phosphorus (SRP) is freely available (>50 µg/L) during and

after intense blooms of diazotrophic cyanobacteria (Lathrop, 2007; McGowan, Leavitt, & Hall, 2005), suggesting that P limitation has not been re-established (Scott & Grantz, 2013). Third, N₂ fixation is energetically demanding (Flores & Herrero, 2005; Herrero, Stavans, & Flores, 2016) and can be limited by low light availability in turbid eutrophic lakes (Ferber et al., 2004; Mugidde, Hecky, Hendzel, & Taylor, 2003). Fourth, nitrogenase enzyme complex activity may be limited by supply of micronutrients (Howarth, Marino, Cole, Lane, & Howarth, 1988; Marino, Howarth, Chan, Cole, & Likens, 2003). Fifth, fixed N is readily released from diazotrophic cyanobacteria (Glibert & Bronk, 1994; Patoine et al., 2006) and can be transformed and returned to the atmosphere via microbial denitrification before uptake by other phytoplankton, particularly in eutrophic lakes (Bruesewitz, Hamilton, & Schipper, 2011; David, Wall, Royer, & Tank, 2006; Seitzinger et al., 2006). These diverse observations, in conjunction with the observation that lakes differ by up to 100-fold in the availability of water-column P (Harke et al., 2016; Patoine et al., 2006), prohibit generalisation about the importance of fixed N₂ in sustaining lake production and P limitation.

In this study, we used two decades of biweekly experiments and monitoring data from seven lakes to quantify the importance of fixed N to satisfy the biological demand of phytoplankton in eutrophic hardwater lakes characteristic of the northern Great Plains and other continental interiors (Finlay et al., 2015). In addition, we sought to identify the environmental conditions associated with elevated N₂ fixation and importance to phytoplankton. Study sites span a gradient from mesotrophic to hyper-eutrophic lakes that vary in the degree of N and P deficit (Finlay, Patoine, Donald, Bogard, & Leavitt, 2010; Hall, Leavitt, Quinlan, Dixit, & Smol, 1999), N₂ fixation (Patoine et al., 2006), N and P availability (Bogard, Donald, Finlay, & Leavitt, 2012; Donald, Parker, Davies, & Leavitt, 2015) and urban pollution with N (Leavitt et al., 2006). We predicted that fixed N would supply a larger proportion of N demand in perennially N-limited downstream lakes (Smith & Schindler, 2009) and that fixed N would be more important to primary producers in years with elevated production and growth demand (i.e. that production and fixation would vary synchronously; Scott, Stanley, Doyle, Forbes, & Brooks, 2009). Finally, we anticipated that there would be strong longitudinal gradients of N₂ fixation and demand, as downstream eastern lakes in the Qu'Appelle River catchment exhibit lower ratios of N:P (Soranno et al., 1999) and historically higher abundance of diazotrophic cyanobacteria (Patoine et al., 2006; Vogt, Sharma, & Leavitt, 2018). Ultimately, the objective of this study was to ascertain whether retained fixed N is sufficient to meet phytoplankton N demand in P-rich hardwater lakes.

2 | METHODS

2.1 | Study sites

The seven study lakes are located in the Qu'Appelle River drainage basin (*ca.* 52,000 km²) in southern Saskatchewan, Canada (Figure 1) and are part of the Qu'Appelle Long-term Ecological Research

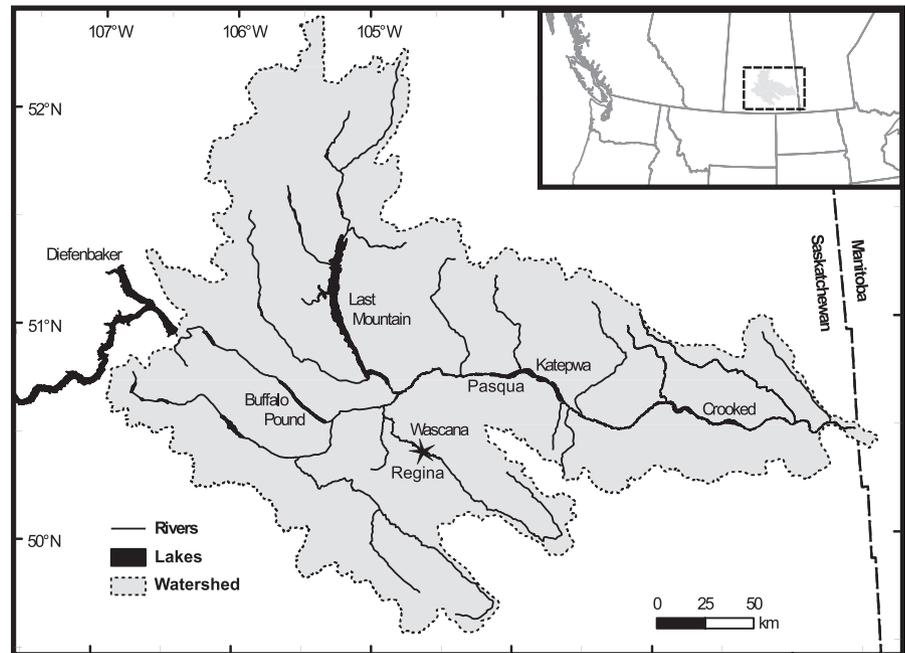


FIGURE 1 Qu'Appelle River drainage basin in southern Saskatchewan (inset), Canada, showing location of study lakes. Watershed is indicated in grey with a dashed line. Wascana Lake is located in an urban park with the City of Regina

network (QU-LTER) as described by Vogt, Rusak, Patoine, and Leavitt (2011), Vogt et al. (2018). The Qu'Appelle River flows eastward from headwaters near Lake Diefenbaker through a series of six productive lakes (including Buffalo Pound, Pasqua, Katepwa and Crooked) to its confluence with the Assiniboine River in Manitoba. Wascana Lake and Last Mountain Lake drain into the Qu'Appelle River mid-reach near the city of Regina. Nitrogen-rich urban effluent from Regina first enters Pasqua Lake before being conveyed to eastern downstream lakes (Katepwa, Crooked). Land use within the Qu'Appelle watershed is comprised mainly of agriculture (75%), along with natural grasslands (12%), the urban centres (5%) of Moose Jaw and Regina, and surface waters (8%; Finlay et al., 2015; Hall et al., 1999). The effective drainage area of each lake, defined as the region supplying water to a lake during years of median river flow, increases from west to east for lakes along the mainstem of the Qu'Appelle River (Figure 1). Southern Saskatchewan's climate is classified as cool-summer humid continental (Köppen Dfb classification), with short summers (mean 19°C in July), cold winters (mean -16°C in January), high evaporation (ca. 60 cm/year) relative to precipitation (ca. 30 cm/year), and ca. 75% of runoff occurring during spring snowmelt (Leavitt et al., 2006; Pham, Leavitt, McGowan, Wissel, & Wassenaar, 2009).

Study lakes range from mesotrophic Lake Diefenbaker to hyper-eutrophic Wascana Lake and vary >10-fold in most physical, chemical and biological properties (Table 1). Organic and inorganic C content is high in all lakes, reflecting regional geology, soil characteristics and high C influx from terrestrial ecosystems (Finlay, Leavitt, Patoine, & Wissel, 2010). Lakes are polymictic in most years, with pronounced deepwater anoxia by late summer in central and eastern lakes (Vogt et al., 2011). Mean summer concentrations of Chl-*a* are elevated in all lakes except mesotrophic Lake Diefenbaker, while ratios of dissolved N:P decline from the mid-reaches of the Qu'Appelle drainage

east to the Manitoba border. Diverse N₂-fixing and non-N₂-fixing cyanobacteria are common in all of the lakes (e.g. *Aphanizomenon*, *Anabaena*, *Gloeotrichia*, *Microcystis*), as are surface blooms during July–September (Donald, Bogard, Finlay, Bunting, & Leavitt, 2013; Finlay, Patoine, et al., 2010; Leavitt et al., 2006).

Summer residence time was calculated for each basin as the lake volume divided by inflow minus evaporation. Groundwater was not included in these calculations as it is expected to be a minor component of the water budget in these lakes. River inflow to each lake was estimated from two sources: gauge-measured flows from data collated by the Government of Canada's Water Office (https://wateroffice.ec.gc.ca/index_e.html) and projections from the Saskatchewan Water Security Agency Water Resources Management Model. Inflow for each lake was calculated as the sum of gauged inflows (May–August). Lake volume was calculated as the average of volume estimates calculated from area capacity curves and daily measurements of lake level.

2.2 | Limnological monitoring

Lakes were sampled biweekly between 1 May (day of year, DOY, 121) and 31 August (DOY 243) following standard protocols (Vogt et al., 2011, 2018). Diefenbaker, Wascana, Last Mountain, Katepwa, and Crooked lakes were sampled 1996–2014, while Pasqua Lake was sampled 2004–2014. Most lakes were sampled between 09:00 hr and 13:00 hr at a single, standard site located at the deepest point of the lake. In contrast, Wascana Lake was sampled at a standard location; however, as the lake was deepened in 2004, this site was the deepest point in the lake only until 2004. Profiles of dissolved oxygen, pH, conductivity and temperature were measured at 1-m intervals using a YSI-85 multi-probe meter (YSI, Inc., Yellow Springs, OH, USA). Depth-integrated water samples were collected by pooling

TABLE 1 Table of lake characteristics

	Diefenbaker	Buffalo Pound	Last Mountain	Wascana	Pasqua	Katepwa	Crooked
Longitude (°W)	106.6	105.5	105.2	104.6	104.0	103.7	102.7
Mean depth (m)	33.0	3.0	7.9	1.5	6.0	14.3	7.9
Lake area (km ²)	500	29	227	0.5	20	16	15
Residence time (per year)	1.3	0.7	12.6	0.02	0.7	1.3	0.5
Secchi (m)	3.4 ± 0.6	1.2 ± 0.6	2.0 ± 0.2	0.8 ± 0.2	1.3 ± 0.3	1.6 ± 0.4	1.4 ± 0.2
Chl- <i>a</i> (µg/L)	5.8 ± 3.4	32.7 ± 13.1	17.7 ± 4.8	40.0 ± 16.8	33.2 ± 10.3	28.6 ± 7.5	30.8 ± 15.3
Chl- <i>a</i> _{surface} : Chl- <i>a</i> _{integrated}	0.78 ± 0.90	1.09 ± 0.32	1.23 ± 0.47	1.08 ± 0.36	1.34 ± 0.27	1.53 ± 0.54	1.40 ± 0.45
TDN (µg/L)	431.4 ± 152.1	532.7 ± 107.6	996.8 ± 100.8	1492.9 ± 317.3	1561.2 ± 495.8	1190.1 ± 268.4	987.0 ± 191.2
DIN (µg/L)	219.9 ± 151.8	134.1 ± 202.1	122.7 ± 168.5	309.0 ± 393.8	780.7 ± 434.9	385.5 ± 333.3	188.7 ± 232.8
SRP (µg/L)	14.9 ± 23.7	40.8 ± 67.6	31.8 ± 26.0	284.8 ± 172.8	139.6 ± 86.3	139.3 ± 77.0	132.4 ± 133.1
TDP (µg/L)	15.6 ± 30.3	29.9 ± 22.6	45.8 ± 51.6	366.9 ± 278.1	165.8 ± 105.9	166.9 ± 73.2	143.7 ± 94.4
Dissolved N:P (mass)	29.0	13.1	31.4	5.2	11.2	8.5	7.5

Parameters include mean (± standard deviation) of samples collected biweekly to estimate Secchi depth, total chlorophyll-*a* (Chl-*a*), ratio of surface to depth-integrated Chl-*a* (Chl-*a*_{surface}: Chl-*a*_{integrated}), total dissolved nitrogen (TDN), dissolved inorganic nitrogen (DIN), soluble reactive phosphorus (SRP), and total dissolved phosphorus (TDP) for the sampled years. See Section 2 for details. Lakes are ordered from west to east in the table.

samples from a 2.2-L Van Dorn water bottle deployed at 0.5-m intervals (Vogt et al., 2011). Depth-integrated water was screened through a 243-µm mesh net to remove zooplankton and stored at 4°C in a dark bottle until processed. An aliquot of 100 ml of depth-integrated water sample was preserved with Lugol's IKI solution for microscopic analysis of the phytoplankton community composition (Donald et al., 2013).

Samples for analysis of Chl-*a*, particulate organic matter (POM), phytoplankton pigments, and stable N isotopes were filtered onto Whatman GF/C glass fibre filters (nominal pore size 1.2-µm), wrapped in aluminum foil, placed in a dark film canister and frozen (-12°C). Depth-integrated water was filtered through 0.45-µm pore size membrane filter and the filtrate was stored until nutrient analysis, as described by Vogt et al. (2011). We estimated primary production using standard dark and light bottle techniques for eutrophic lakes (Rice, Baird, Eaton, & Clesceri, 2012) following Finlay, Leavitt, Wissel, and Prairie (2009). Briefly, triplicate samples of 243-µm screened, depth-integrated lake water were incubated in a growth chamber in either transparent or darkened 250-ml glass bottles. Bottles were incubated at ambient lake temperature and under a 12-hr light/dark cycle with 450 µmol quanta m⁻² s⁻¹, a photon flux comparable to that recorded in situ at Secchi depth using a profiling radiometer (Finlay et al., 2009). Oxygen concentrations in each bottle were measured at the start of the experiment and after incubation for 24 hr.

2.3 | Laboratory analyses

Phytoplankton communities were enumerated to species, and heterocyte abundance was quantified by Dr Ann St. Amand at

PhycoTech, Inc. (St Joseph, MI, USA). Briefly, an Olympus BHT/BMX light microscope was used to identify, enumerate and measure the linear dimensions of individual cells or colonies and heterocytes of each species using the Utermöhl technique (Utermöhl, 1958). Heterocyte enumeration was restricted to samples collected over 7 years from Crooked and Katepwa lakes, the two basins with the highest rates of N₂ fixation in preliminary studies (Patoine et al., 2006).

Chlorophyll-*a* samples were analysed using standard trichromatic spectrophotometric methods (Rice et al., 2012). Briefly, Chl-*a* was extracted from the filters with 80% acetone: 20% methanol and the wavelength-specific absorbance was quantified using a Hewlett Packard model 8452A photo-diode array spectrophotometer (1996–2004) or an Agilent model 8453 UV-Visible spectrophotometer (2005–2014). Chlorophyll-*a*, carotenoid, and derivative pigments from phytoplankton were quantified using the standard high-performance liquid chromatography (HPLC) techniques of Leavitt and Hodgson (2001). Pigments were extracted from filters with extraction solvent before completely drying under N₂ gas. Dried pigments were stored under an inert N₂ atmosphere then redissolved into an injection solution before introduction into the HPLC. Concentrations of biomarker pigments (nmoles pigment/L) were estimated for compounds characteristic of total algal abundance (Chl-*a*, pheophytin *a*, β-carotene), chlorophytes and cyanobacteria (lutein-zeaxanthin), total cyanobacteria (echinenone), colonial cyanobacteria (myxoxanthophyll), and Nostocales cyanobacteria (canthaxanthin; Leavitt & Hodgson, 2001). In this HPLC system, structural isomers from chlorophytes (lutein) and cyanobacteria (zeaxanthin) were not separated and were instead used to estimate abundance of summer bloom-forming taxa (Hall et al., 1999; Leavitt

et al., 2006). The proportion of phytoplankton biomass in the surface waters (surface to integrated Chl-*a*) was calculated by dividing the Chl-*a* from a surface grab sample by the Chl-*a* concentrations of a depth-integrated water sample.

The elemental composition (%C, %N, C:N mass ratio) and stable nitrogen isotope value ($\delta^{15}\text{N}$) of particulate organic material (POM) from depth-integrated water samples were estimated by combustion and isotope-ratio mass spectrometry following Savage, Leavitt, and Elmgren (2004). POM was scraped from frozen filters into pre-weighed tin capsules, dried to constant weight, and combusted in a NC2500 Elemental Analyzer (ThermoQuest; CE Instruments) coupled to a Thermoquest (Finnigan-MAT) Delta Plus^{XL} IRMS. Nitrogen stable isotope ratios are reported in the conventional δ notation with respect to atmospheric N_2 .

Nutrient content of lake waters was estimated using standard QU-LTER protocols at the Biogeochemical Analytical Service Laboratory, University of Alberta, Edmonton, Alberta, Canada (Vogt et al., 2011, 2018). Depth-integrated water was screened (243- μm mesh) then filtered through an 0.45- μm pore size membrane filter within 3 hr of collection before analysis for total dissolved phosphorus and orthophosphate (SRP), both as $\mu\text{g P/L}$, as well as NO_3^- , NH_4^+ , and total dissolved nitrogen (TDN), which includes organic and inorganic fractions of dissolved nitrogen (all mg N/L) using standard analytical procedures (Stainton, Capel, & Armstrong, 1977).

2.4 | Estimation of N demand and N_2 fixation

Nitrogen demand was calculated from bioassay estimates of phytoplankton productivity ($\text{mg C fixed m}^{-3} \text{hr}^{-1}$), in situ estimates of light penetration (Finlay et al., 2009), measured day-length, and estimates of phytoplankton C:N ratios from POM samples (Finlay, Leavitt, et al., 2010; Patoine et al., 2006). This approach assumed that: there was a constant stoichiometry between the rate of oxygen evolution and carbon fixation (Sala, Jackson, Mooney, & Howarth, 2000); phytoplankton acquired sufficient N to meet the measured rate of C uptake and maintain observed cellular ratios of C:N (Ferber et al., 2004); and POM was composed mainly of phytoplankton (Donald et al., 2013). Microbial respiration (R) was estimated as the rate of decline in oxygen in the dark bottle, whereas net primary productivity (NPP) was estimated from increased oxygen concentrations in light bottles, and gross primary productivity (GPP) was calculated as the sum of NPP and R (all as $\text{mg O m}^{-3} \text{hr}^{-1}$). We assumed that R includes both autotrophic and heterotrophic respiration by microbes and that R in light and dark were similar, such that GPP represented mainly autotrophic processes (Rice et al., 2012). Metabolic rates were converted to carbon equivalents ($\text{mg C m}^{-3} \text{hr}^{-1}$) assuming a photosynthetic quotient of 1.24 (Sala et al., 2000). Nitrogen demand ($\text{mg N m}^{-3} \text{hr}^{-1}$) was estimated by multiplying GPP by the C:N of POM, assuming that phytoplankton maintained their cellular N quota at the observed C:N ratios. We used GPP to estimate N demand because dark and light bottle assays more accurately estimate GPP than NPP and because bottle-based estimates of GPP

correlated strongly with phytoplankton standing stock in these lakes (Finlay et al., 2009).

Whole-lake and summer-long estimates of N demand in these polymictic lakes were determined from biweekly estimates of lake transparency, day length, and gross planktonic productivity described above (Finlay, Leavitt, et al., 2010; Finlay et al., 2009). Estimates of whole-lake planktonic N demand were calculated by multiplying lab-based rates of N demand with the fraction of the day spent in the euphotic zone, defined as the ratio of the euphotic volume (as Secchi depth) to total lake volume. We assumed that our summer-long whole-lake estimates of phytoplankton productivity captured a high proportion of spatial and temporal variation in GPP attributable to changes in light regimes. We made this assumption because incubator irradiance was equivalent to that measured at Secchi depth, Secchi depth varied seasonally from ca. 10 cm to ca. 8 m, and rates of photosynthesis are typically independent of irradiance at higher photon fluxes (MacIntyre, Kana, Anning, & Geider, 2002). Further discussion of these assumptions, and their comparison to other methods of estimating lake production, is presented in Finlay, Leavitt, et al. (2010) and Finlay et al. (2009).

Two approaches were used to estimate rates of nitrogen fixation: we compare the more commonly used heterocyte-based method (Finlay et al., 1994) to demonstrate the accuracy of our isotope-based method (Patoine et al., 2006). First, we used two forms of the natural abundance method (NAM) developed for both freshwater ecosystems (Patoine et al., 2006) and oceanographic studies (Karl et al., 1997). NAM mixing models assume that summer declines in the $\delta^{15}\text{N}$ of lake nitrogen arise mainly from N_2 fixation, a process that is offset, in part, by a similar magnitude of isotopic enrichment due to denitrification (Leavitt et al., 2006; Patoine et al., 2006; Peterson & Fry, 1987). As such, NAM integrates N transformations and represents the residual flux (gross N_2 fixation - denitrification) of fixed N to primary producers. This is the equivalent to the proportion of total fixed N that remains in the water column after denitrification and is referred to as net N_2 fixation hereafter. Because over 75% of total annual inflow occurs some 4–7 weeks prior to monitoring (Pham et al., 2009), we assumed that in each year most allochthonous N had already entered the lake by the start of our estimates of N fixation or demand (Patoine et al., 2006; Pham et al., 2009) and that phytoplankton mainly used this dissolved N pool, which includes both inorganic and organic forms of nitrogen, for growth.

In the first NAM method, and throughout this paper, we modelled the influx of N into the particulate pool by measuring changes in $\delta^{15}\text{N}$ of POM following Patoine et al. (2006). In addition, we compared these results to a second NAM method that modelled the influx of fixed N into the TDN pool by measuring changes in $\delta^{15}\text{N}$ of solutes isolated from whole water by freeze-drying aliquots of water that had been filtered through GFC (1.2- μm pore) and membrane filters (0.45- μm pore) as described in the Supporting Text. Unless noted, the N_2 -fixation values presented in text are the result of the POM-based calculations. Although we modelled the summer period when river inflow was a small part of the hydrological budget (Dröscher,

Patoine, Finlay, & Leavitt, 2009; Pham et al., 2009), we tested for the potential effects of hydrological influx of allochthonous N (Jankowski, Schindler, & Holtgrieve, 2012) by quantifying the statistical relationships between inflow, residence time, and N fixation or demand.

For each lake and year, linear regression was used to describe the relationship between seasonal changes in the $\delta^{15}\text{N}$ of POM (or TDN) on a standardised DOY near the start ($\delta_t = \text{DOY } 130$) and end ($\delta_{t+1} = \text{DOY } 234$) of each year's sampling period. For this calculation, we assumed that the $\delta^{15}\text{N}$ value of the final ($\delta_{t+1}^{15}\text{N}$) is the mass-weighted average of $\delta_t^{15}\text{N}$, the initial mass of POM-N (or TDN), and the isotopic value of N_2 -fixing cyanobacteria (δ_{fix} ; 0‰; Zhang, Sigman, Morel, & Kraepiel, 2014):

$$\delta_{t+1} = \frac{M_t \delta_t + M_{\text{fix}} \delta_{\text{fix}}}{M_t + M_{\text{fix}}}, \quad (1)$$

where M_t and M_{fix} represent the standing stock mass of POM-N (or TDN) at DOY 130 and the amount of fixed N (both as Mg N), respectively. We calculated POM-N standing stock first by converting total phytoplankton biomass (measured as Chl-*a* concentration) to carbon by multiplying by a standard Chl-*a* to carbon conversion ratio (Patoine, Graham, & Leavitt, 2006). We converted from POM-C to POM-N by multiplying by POM C:N then multiplied by whole-lake volume to estimate the standing stock. The fractional increase in standing stock attributable to N_2 fixation was then calculated as:

$$M_{\text{fix}}/M_t = \frac{\delta_t - \delta_{t+1}}{\delta_{t+1} - \delta_{\text{fix}}}, \quad (2)$$

before the total mass of fixed N was estimated from standing stock of POM-N (PON_{ss} ; or TDN):

$$M_{\text{fix}} = \left(\frac{M_{\text{fix}}}{M_t} \right) \times \text{PON}_{\text{ss}}. \quad (3)$$

Mean summer rates of retained fixed N accumulation ($\text{mg N m}^{-3} \text{ hr}^{-1}$) were calculated from estimates of whole-summer fixed mass by dividing the summer total by the product of lake volume and the number of daylight hours in the growing season (mean 12 hr/day and 104 days/year).

In the second approach to estimate N_2 fixation, gross rates of N_2 fixation by cyanobacteria were calculated by applying in vitro estimates of heterocyte-specific N_2 fixation rates to microscopic determinations of in situ heterocyte density in Katepwa and Crooked lakes, following procedures developed at the Experimental Lakes Area (ELA) (Findlay et al., 1994; Higgins et al., 2017). Cell-specific and volumetric (per μl) rates of N_2 fixation were obtained from a comprehensive search of the literature of studies that quantified cyanobacterial N_2 fixation under natural conditions (Supporting Information Table S1), and were slightly higher than those used at ELA (Findlay et al., 1994). This mean ($n = 7$) heterocyte-specific rate ($8.845 \times 10^{-17} \text{ g N heterocyte}^{-1} \text{ s}^{-1}$) was applied to biweekly enumerations of heterocyte density in Crooked and Katepwa lakes, sites that were known to have elevated densities of N_2 -fixing cyanobacteria (Leavitt et al., 2006; Patoine et al., 2006). As shown at ELA, such heterocyte-based methods provide estimates

of N_2 fixation that are a strong linear function ($r^2 > 0.90$) of those derived from the commonly used acetylene reduction protocol and newer ^{15}N uptake techniques (Findlay et al., 1994; Higgins et al., 2017).

Finally, estimates of N_2 fixation from both NAM and heterocyte approaches were compared to each other and to literature values to assess potential bias. In this case, we focused on estimates of N_2 fixation in eutrophic systems most similar to lakes of the Qu'Appelle River drainage, as well as unpublished estimates from our study lakes based on ^{15}N protocols.

2.5 | Numerical analyses

Candidate predictor variables to be used in modelling N processes were selected based on 25 years of previous research in these systems (Finlay, Patoine, et al., 2010; Hall et al., 1999; Leavitt et al., 2006; Vogt et al., 2018). Predictor variables included metrics of total phytoplankton biomass (trichromatic Chl-*a*, β -carotene), cyanobacteria-specific biomarker pigments (canthaxanthin, echinenone), dissolved nutrients (NH_4^+ , NO_3^- , TDN, total dissolved phosphorus and SRP), seasonal climate indices (Pacific Decadal Oscillation, El Niño–Southern Oscillation), and seasonal residence time. We computed Pearson's correlation coefficients, r , between all pairs of predictor variables and retained only those with $r \leq 0.2$; the predictor variable with the clearest mechanistic link to N fixation was retained in the final models. Candidate predictors were $\log_{10}(x + 1)$ -transformed to normalise variance, as needed.

Trends in our estimates of fixed N were modelled with a generalised additive model (GAM) and thin plate spline bases using the *mgcv* package (Wood, 2011) in R version 3.3.3 (R Core Team, 2017). We chose to model the data using a GAM because this approach better accounts for non-linearity in modelled relationships (as well as non-monotonic relations), while also allowing us to quantify uncertainties. The initial model included total phytoplankton biomass (as trichromatic Chl-*a*), a biomarker pigment of Nostocales cyanobacteria (canthaxanthin), TDN, TDN:SRP, the winter (January–March) index of the Pacific Decadal Oscillation climate system, and summer residence time (May–August), with lake identity included as a random intercept. Explanatory variables were removed from the model via shrinkage based on restricted maximum likelihood (REML) smoothness selection and the double-penalty method (Marra & Wood, 2011). The analysis was made robust to outliers by assuming that the response variable followed a scaled t -distribution rather than a Gaussian distribution (Wood, Pya, & Säfken, 2016). Extreme canthaxanthin concentrations were removed from annual time series to improve spline fits in 2 lake-years.

Analysis of temporal coherence was used to assess whether N demand and estimates of fixed N were responding to similar environmental drivers. Synchrony, S , was estimated as the average of all pair-wise Pearson correlations between lakes and years, with values ranging from 1 (perfectly and positively coherent) to -1 (perfectly

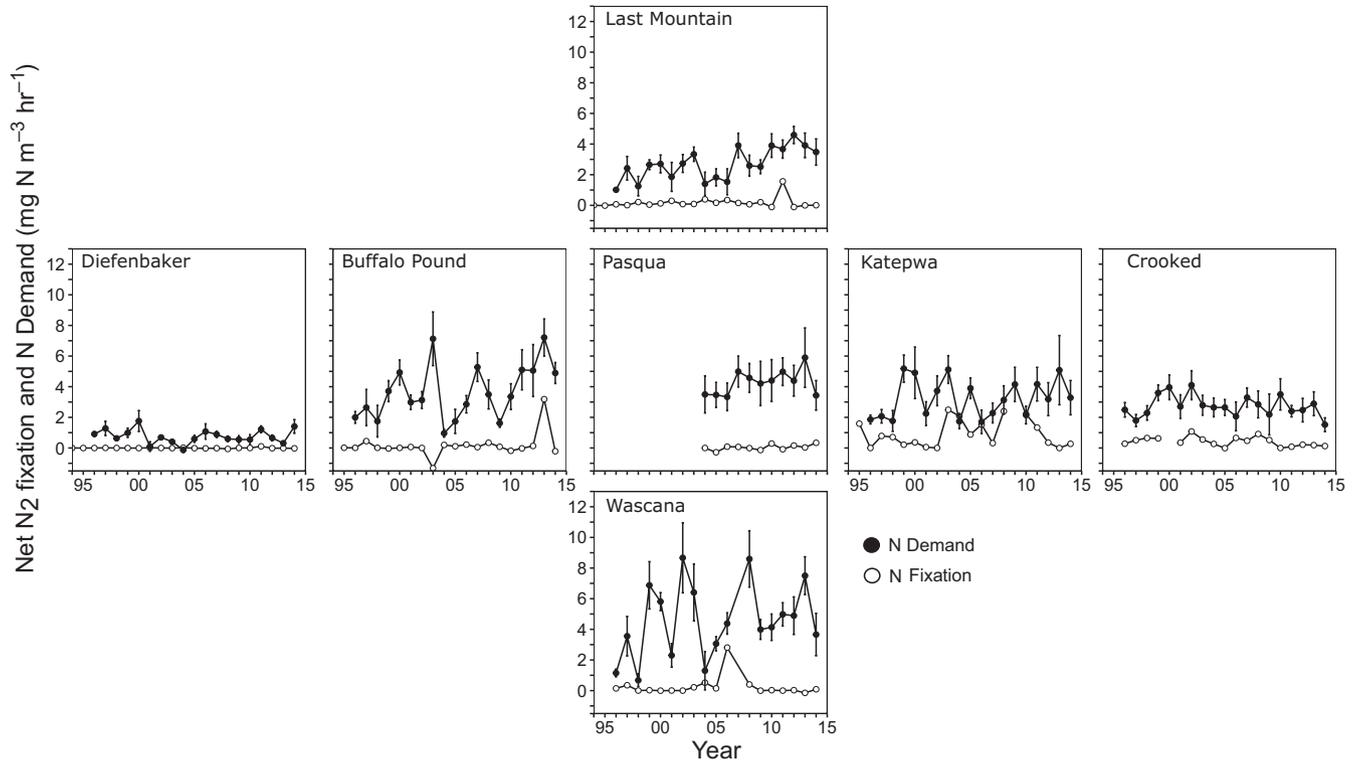


FIGURE 2 Mean (\pm standard deviation) summer phytoplankton N demand (filled circle) and N_2 fixation (open circle) for seven study lakes ($\text{mg N m}^{-3} \text{hr}^{-1}$) using the POM NAM method. Lakes are presented along the water flowpath (Figure 1) from upstream Lake Diefenbaker to downstream Crooked Lake with Last Mountain and Wascana lakes draining into the Qu'Appelle River via tributary streams upstream of Pasqua Lake. Each point is the mean of 8 biweekly samples collected during May–August each year

synchronous but out of phase) through 0 (completely asynchronous). Comparison of S values among a diverse set of environmental, physico-chemical and biological parameters in these lakes demonstrates that variables with similar degrees of temporal coherence are often subject to common regulatory mechanisms (Vogt et al., 2011). All statistical analyses were performed using R version 3.3.3 (R Core Team, 2017).

3 | RESULTS

3.1 | N demand and retained fixed N

Mean summer algal N demand varied by two orders of magnitude between <0.1 and $9.2 \text{ mg N m}^{-3} \text{hr}^{-1}$ over 117 lake-years of study (Figure 2 and Supporting Information Figure S1). On average, N demand was significantly lower in the mesotrophic Lake Diefenbaker than in any of the downstream eutrophic lakes (Kruskal–Wallis $\chi^2 = 54.97, p = .001$). Nitrogen requirements of phytoplankton did not differ significantly among the six downstream lakes, in part because of a high degree of interannual variability in N demand. Demand for N was moderately synchronous when considering all lakes ($S = 0.41$), and exhibited slightly higher temporal coherence when Pasqua Lake was removed from analysis ($S = 0.48$). Pasqua Lake receives effluent from the city of Regina wastewater treatment plant and, as a result, receives point source N-pollution year-round in addition to

the seasonal flux of diffuse N that all lakes receive. Synchrony did not arise from trends in N demand, as only Last Mountain exhibited an increase over the study period ($r^2 = .45, p \ll .001$; Supporting Information Table S2).

Fixed N, calculated with the POM-based method, was retained by phytoplankton biomass (net N_2 fixation) in 75.2% of the lake-years (88 of 117), while N efflux (denitrification $>$ fixation) was recorded in the other years (Figure 2). Estimates of N_2 fixation rates ranged four orders of magnitude from 0.002 to $3.2 \text{ mg N m}^{-3} \text{hr}^{-1}$, with higher N influx observed in downstream Crooked and Katepwa lakes. Mean rates of N_2 fixation were lower in mesotrophic Lake Diefenbaker ($\chi^2 = 43.07, p < .01$) where net N was apparently lost in 12 years (i.e. negative N_2 fixation). Similarly, N-polluted Pasqua Lake experienced an apparent net N efflux in 4 of 11 years, whereas atmospheric N release was observed only once each in downstream Katepwa and Crooked lakes.

Comparison of the N_2 fixation rates by POM with estimates of N demand by pelagic primary producers revealed that fixed N accounted for a median of 3.5% (mean \pm standard deviation; $11.5 \pm 21.6\%$) of phytoplankton requirements across all Qu'Appelle study lakes (Figure 3). Fixed N was a trivial fraction of phytoplankton N requirements in mesotrophic Lake Diefenbaker (mean $< 0.0\%$) and N-polluted Pasqua Lake (mean = 1.1%). In contrast, fixed N contributed between 15.0% (Crooked) and 36.3% (Katepwa) of the total planktonic N demand in downstream eutrophic lakes. In these

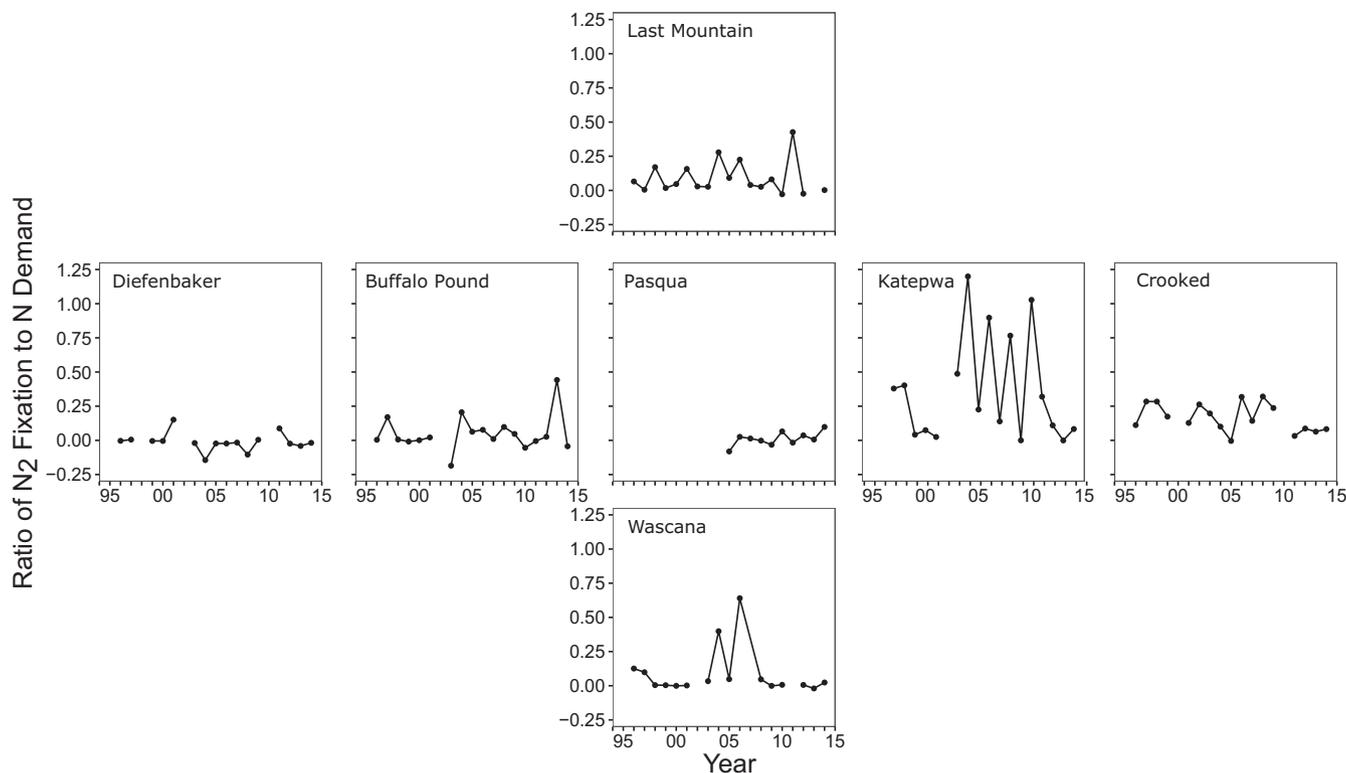


FIGURE 3 Ratio of mean summer N_2 -fixation to phytoplankton nitrogen demand (both $\text{mg N m}^{-3} \text{hr}^{-1}$) for May–August of each year. Values were positive when N demand was greater than N_2 -fixation and values were negative when N_2 -fixation was negative, potentially indicating greater denitrification than N_2 -fixation. Lake organisation as Figure 2

systems, net N_2 fixation was recorded in 30 of the 42 lake-years while, in 4 years, fixed N appears to supply all of phytoplankton requirements (Katepwa Lake only). In the three remaining lakes (Buffalo Pound, Last Mountain, Wascana), fixed N represented an intermediate proportion of N demand (mean = 4.9%–9.1%), even though shallow Wascana Lake is hyper-eutrophic, strongly N-limited in summer, and contained mainly colonial cyanobacteria after June in each year (Donald et al., 2013; Finlay, Patoine, et al., 2010; McGowan et al., 2005). Excluding N-polluted Pasqua Lake, there was a significant increase in the importance of fixed N to phytoplankton nutrition with lake longitude ($\alpha = 0.1$), with a greater magnitude of fixed N in downstream eastern lakes (Supporting Information Figure S2).

Lake-specific estimates of the mass of fixed N varied from 0.0% to 12.2% of spring standing stock of N (Figure 4). Overall, fixed N was a greater proportion of N standing stock during spring in downstream Katepwa (mean = 12.2%) and Crooked lakes (11.5%), with a significant relationship ($r^2 = 0.59$, $p = 0.07$) with landscape position if Pasqua is excluded (Figure 4 and Supporting Information Table S2). Fixed N represented the lowest fraction of vernal N pools in mesotrophic Lake Diefenbaker (0.0%) and N-polluted Pasqua Lake (0.3%). Despite significant increases in spring N stock through time in Last Mountain, Pasqua, Katepwa and Crooked lakes (Supporting Information Figure S3), only easternmost Crooked Lake exhibited a progressive decline in the fraction of spring N attributable to retained fixed N (Supporting Information Table S2).

3.2 | Environmental predictors of N_2 fixation

Only in situ abundance of Nostocales cyanobacteria (as canthaxanthin) was retained as a significant ($p < 0.001$) predictor of the rates of N_2 fixation in the final GAM (Figure 5). GAM analyses showed that N_2 fixation rates increased in a saturating relationship with the water-column concentration of canthaxanthin, although there was high variation in the estimated relationship at elevated Nostocales abundances (Figure 5). No significant N_2 fixation was recorded when canthaxanthin was absent from the phytoplankton pigment assemblage.

3.3 | Comparison of isotopic- and heterocyte-based estimates of N_2 fixation

Heterocyte abundance varied with season and year in both Katepwa and Crooked lakes (Figure 6). Heterocytes were present in all years, particularly after July (data not shown), with similar mean (\pm standard deviation) summer densities in Katepwa (0.31 ± 0.58 heterocytes/L) and Crooked basins (0.76 ± 2.9 heterocytes/L). Rates of gross N_2 fixation based on enumerated in situ heterocyte density and physiological estimates of heterocyte activity (Supporting Information Table S1) ranged from 0.02 to $1.78 \text{ mg N m}^{-3} \text{hr}^{-1}$ ($0.30 \pm 1.14 \text{ mg N m}^{-3} \text{hr}^{-1}$), slightly lower than that observed for the NAM approaches during the same time intervals ($0.66 \pm 70 \text{ mg N m}^{-3} \text{hr}^{-1}$, range -0.01 to $2.40 \text{ mg N m}^{-3} \text{hr}^{-1}$). In most lakes, NAM estimates of N_2 fixation based on changes in the mass and isotopic composition of whole

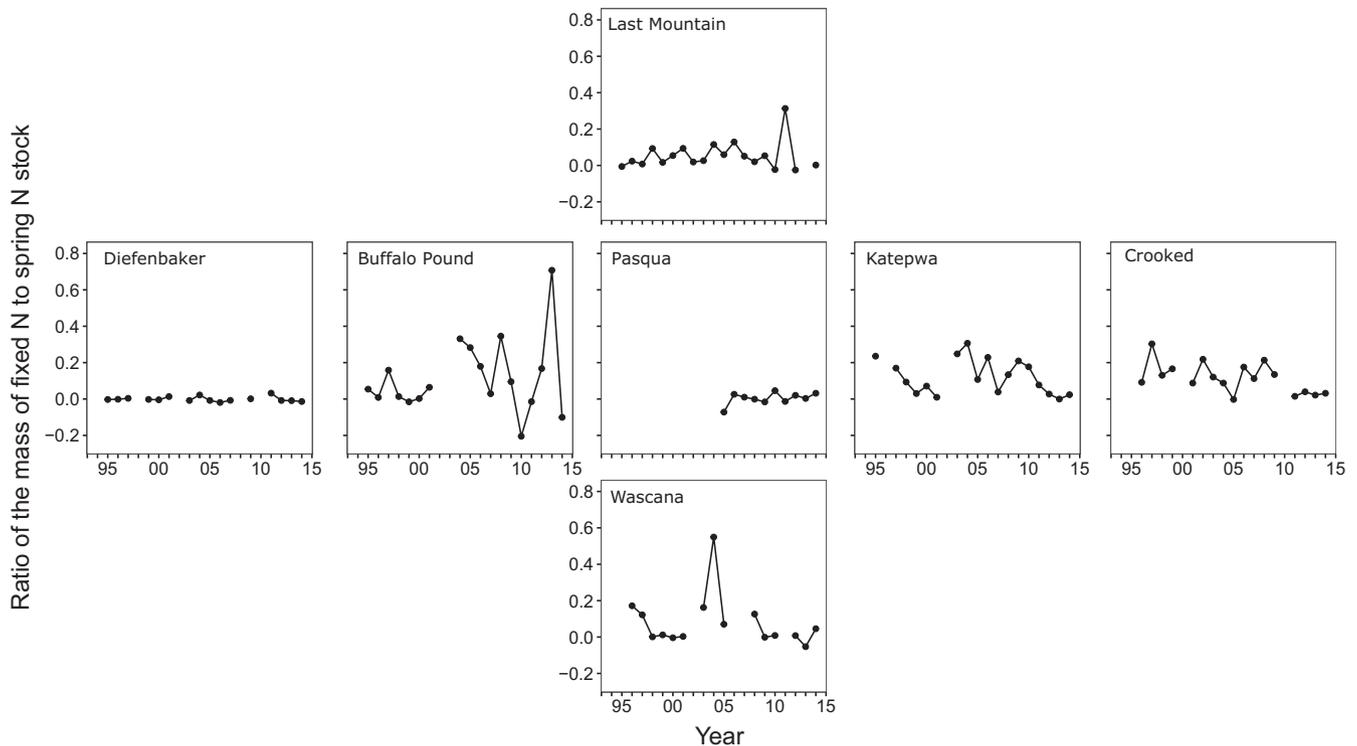


FIGURE 4 Ratio of the total mass of fixed N_2 (kg/summer) to total dissolved N in each lake on first sampling date in May. Values were positive when N demand was greater than N_2 -fixation and values were negative when N_2 -fixation was negative, potentially indicating greater denitrification than N_2 -fixation. Lake organisation as in Figure 2

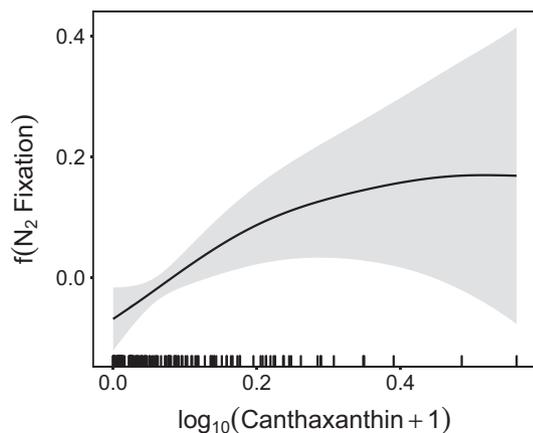


FIGURE 5 Partial response plot of generalised additive model illustrating the relationship between abundance of potentially N_2 -fixing Nostocales cyanobacteria as biomarker pigment canthaxanthin and rate of net N_2 fixation ($\text{mg N m}^{-3} \text{ hr}^{-1}$). The black line illustrates the smoothed relationship between the variables and the gray band is a 95% across-the-function confidence interval. Tick marks on the X-axis correspond to canthaxanthin concentrations of data points included in the model

filtered water were lower than those based on POM, but were similar to heterocyte-derived determinations in Katepwa and Crooked lakes (Supporting Information Figure S4).

Supply of fixed N to primary producers did not appear to re-establish P limitation of the phytoplankton. For example,

phytoplankton biomass was correlated positively ($r = .42$, $p < .001$) with concentrations of SRP, indicating that primary producers did not deplete SRP pools in summer (Supporting Information Figure S5a). Similarly, the degree of phytoplankton growth limitation by P in bioassays was correlated negatively ($r = -.32$, $p < .001$) with Chl-*a* concentrations (Supporting Information Figure S5b), with little evidence of strong P limitation over a wide range of Chl-*a* values (10–100 $\mu\text{g/L}$). Finally, there was no relationship between rates of N_2 fixation and mean annual SRP, mean spring SRP, annual summer biomass, or mean summer P limitation status from bottle bioassays (Supporting Information Figure S5c–f).

4 | DISCUSSION

The role of fixed N in meeting phytoplankton demand, alleviating seasonal N limitation and re-establishing P limitation in lakes is contentious in modern limnology (Paerl et al., 2016; Schindler et al., 2008, 2016; Scott & McCarthy, 2010). Although N_2 fixation meets planktonic N demands in a small boreal lake fertilised with moderate concentrations of P (Findlay et al., 1994; Higgins et al., 2017), to this point, it has been unclear whether diazotrophic processes can meet pelagic demands in systems with up to 100-fold higher concentrations of TP (Bunting et al., 2016; Leavitt et al., 2006). Here we demonstrate that estimates of fixed N (Figures 2, 6) represent a median of 3.5% (mean 11.5%) of the nutritional N requirements of phytoplankton in 117 of lake-year of study (Figures 2, 3). The magnitude

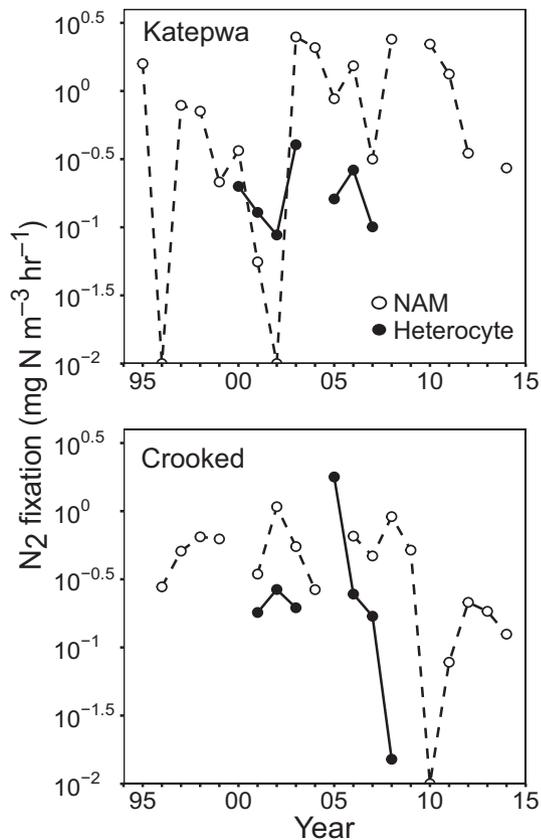


FIGURE 6 Comparison of mean summer estimates of net N_2 fixation rate in Katepwa and Crooked lakes calculated using natural abundance stable isotope method (open circle, dashed line) and estimates of gross N_2 fixation ($\text{mg N m}^{-3} \text{hr}^{-1}$) derived from observed in situ heterocyte density (heterocytes/ m^3) and physiological estimates of heterocyte-specific fixation (filled circle, solid line; $\text{g N m}^{-3} \text{heterocyte}^{-1} \text{s}^{-1}$). See Supporting Information Methods and Table S1 for additional details

of fixed N varied asynchronously among lakes ($S = -0.03$), independent of regionally-coherent phytoplankton demand ($S = 0.41$), but in proportion to changes in abundance of Nostocales cyanobacteria (Figure 5), as seen elsewhere (Ferber et al., 2004; Hendzel, Hecky, & Findlay, 1994; Howarth, Marino, Cole, et al., 1988; Scott & Grantz, 2013; Smith, 1983). Although rates of fixed N were comparable to gross N_2 fixation estimates recorded in other eutrophic lakes (Howarth, Marino, Lane, & Cole, 1988; Vrede et al., 2009), diazotrophic N supply was only a small fraction of phytoplankton demand, and was insufficient to deplete water-column concentrations of SRP during summer blooms or initiate P limitation of phytoplankton growth (Donald, Bogard, Finlay, & Leavitt, 2011; Donald et al., 2015; Finlay, Patoine, et al., 2010).

4.1 | Magnitude of N_2 fixation by phytoplankton

Mean estimates of fixed N accumulation based on NAM approaches ($0.31 \pm 0.64 \text{ mg N m}^{-3} \text{hr}^{-1}$) were similar to values observed for gross N_2 fixation determined for eutrophic lakes using acetylene reduction techniques (Higgins et al., 2017; Scott & Grantz, 2013; Torrey

& Lee, 1976; Vrede et al., 2009), heterocyte densities (Findlay et al., 1994; Higgins et al., 2017; Schindler et al., 2008), and ^{15}N bioassay approaches conducted recently in Qu'Appelle lakes ($0.04\text{--}4.45 \text{ mg N m}^{-3} \text{hr}^{-1}$; L. Boyer and H. Baulch, University of Saskatchewan, unpublished data). NAM techniques integrate N transformations across all lake habitats, as would be important in polymictic eutrophic lakes with intense microbial N transformations (Bruesewitz et al., 2011; David et al., 2006; Seitzinger et al., 2006), including N_2 fixed by heterotrophic organisms (Howarth, Marino, Lane, & Cole, 1988) and non-heterocystous fixation (Bergman, Gallon, Rai, & Stal, 1997). Further work is needed to refine estimates of N_2 fixation in aquatic ecosystems (Jankowski et al., 2012), including validation of the acetylene reduction protocols, which is known to inhibit the action of nitrogenase (Fulweiler et al., 2015). However, the close similarity of results from two NAM protocols, a heterocyte-based method, and literature estimates of acetylene reduction (Supporting Information Figure S4) strongly supports the conclusion that fixed N is only a small fraction of phytoplankton N demand that does not re-establish P limitation in these SRP-rich study lakes.

4.2 | Importance of fixed N to phytoplankton and lakes

Increased interest in the importance of fixed N in lake ecosystems arises in part because of current debate concerning the need to regulate N and P pollution to control eutrophication (Paerl et al., 2016; Schindler et al., 2016). In boreal lakes, N_2 fixation by planktonic cyanobacteria appears to compensate for experimental reduction in N influx (Schindler et al., 2008; Paterson et al., 2011 but see Scott & McCarthy, 2010, 2011). However, in other regions, N influx has been demonstrated to increase lake production up to four-fold in low- and replete-P lakes, alike (Bunting et al., 2007; Deiningner, Faithfull, & Bergström, 2017; Leavitt et al., 2006). In particular, our findings suggest that despite abundant diazotrophic cyanobacteria (McGowan et al., 2005; Patoine et al., 2006; Vogt et al., 2018) and rates of fixed N retention similar to fixation rates typical of other eutrophic lakes (Howarth, Marino, Lane, & Cole, 1988; Scott & Grantz, 2013; Vrede et al., 2009), biological N_2 fixation may not alleviate N limitation in P-rich lakes (Finlay et al., 2015). In these lakes, the combination of geological substrate (Hall et al., 1999; Klassen, 1989) and over 75 years of agricultural fertilisation with P (Bennett et al., 2001; Carpenter, 2005) leads to elevated P export to lakes and rivers (Bennett et al., 2001; Carpenter et al., 1998; Stoddard et al., 2016) and has resulted in TP and SRP levels 100-fold greater than in other lake regions (Leavitt et al., 2006; Vogt et al., 2018).

Several lines of evidence suggest that rates of N_2 fixation were insufficient to satisfy N demands in most years in these productive lakes (Figures 2, 3, 6). First, fixed N represented only 3.5% of total N supply to phytoplankton (Figure 3) and <5% of spring N standing stock (Figure 4) in most lakes and years, consistent with previous whole-lake mass budgets (Donald et al., 2015; Leavitt et al., 2006; Patoine et al., 2006) and enriched $\delta^{15}\text{N}$ values of POM in all lakes (Supporting Information Figure S6). As noted by Ferber et al. (2004), N isotope values of POM are depleted to approximately atmospheric

values (0‰ to -2‰) when fixed N_2 is the main source of cellular N, but not when other sources of N are paramount. Second, water-column concentrations of dissolved P were not depleted despite accumulation of fixed N in the phytoplankton (Table 1, Supporting Information Figure S5). Instead, SRP concentrations remained elevated throughout July and August in all basins except Lake Diefenbaker, the period when Chl-*a* and diazotrophic cyanobacteria are abundant (Donald et al., 2011; Finlay, Patoine, et al., 2010; Patoine et al., 2006). Third, net N efflux (N losses exceed N_2 fixation) was common only in headwaters (Figure 3), consistent with the P-limited status of upstream Diefenbaker and Buffalo Pound lakes (Abirhire et al., 2015; Quiñones-Rivera, Finlay, Vogt, Leavitt, & Wissel, 2015; Vogt et al., 2018). Finally, the absence of trends in time series of either total mass of fixed N (Figure 2) or proportion of phytoplankton demand (Figure 3) for most lakes (Supporting Information Table S2) suggests that over 50 years of N_2 fixation (Hammer, 1971; Patoine et al., 2006) has been insufficient to alter the nutrient status of these lakes.

N_2 fixation alone was sufficient to completely meet total phytoplankton N demands in ca. 20% of surveyed years in Katepwa Lake, and commonly met 25% of requirements in eutrophic Crooked Lake (Figures 2, 3). However, in most other instances, including all but 4 years in hyper-eutrophic Wascana Lake, N_2 fixation rarely exceeded 5% of planktonic N demand, a value which was often statistically-indistinguishable from zero (Supporting Information Figure S7). Given the role of Nostocales cyanobacteria in the prediction of N_2 fixation (Figure 6 and below), we infer that the nutritional demands of the phytoplankton are met by atmospheric N_2 only during years in which limnological conditions favoured extreme densities of colonial diazotrophic species (see also Patoine et al., 2006). In principle, Nostocales and other potentially N_2 -fixing cyanobacteria are promoted by elevated water temperature (Jöhnk et al., 2008; Paerl & Scott, 2010), intense grazing by large-bodied cladocerans (Elser et al., 2000), low flushing rates (Elliott, 2010), sufficient micro-nutrient availability (Howarth, Marino, Lane, & Cole, 1988; Marino et al., 2003), elevated light regimes (Ferber et al., 2004; Mugidde et al., 2003) and low N:P ratios (Levine & Schindler, 1999; Smith, 1983). In general, densities of colonial cyanobacteria in Qu'Appelle lakes are correlated positively to surface water temperature and negatively to wind speed and the El Niño–Southern Oscillation index (Vogt et al., 2018), although the high proportion of unexplained variance (>40%) suggests a high degree of site-specific variation (Dröscher et al., 2009). Taken together, these findings suggest that while N_2 fixation is rarely a substantial source of N to natural phytoplankton assemblages in Qu'Appelle lakes (Figures 2, 3), factors that selectively increase surface blooms of cyanobacteria may increase the importance of fixed N_2 in the future (Paerl & Scott, 2010).

Cross-validation with multiple methods shows that estimates of N demand and fixed N are robust for the study systems. Our bottle assays of GPP are based on standard protocols used in eutrophic systems for >70 years (Rice et al., 2012) and provide a reliable metric of lake productivity similar to those derived from whole-lake C budgets (Finlay, Leavitt, et al., 2010), CO_2 flux estimates derived

from gaseous stable isotopes (Quiñones-Rivera et al., 2015), and Chl-*a* based estimates of phytoplankton standing stock (Finlay et al., 2009; McGowan et al., 2005). Similarly, community analysis using HPLC and microscopic enumeration provide highly correlated estimates of phytoplankton abundance (Donald et al., 2013) suggesting that detrital material is not a substantial component of POM in these lakes. Often, C:N ratios are elevated in detrital material (Moe et al., 2005), reflecting the preferential mineralisation of N from dead phytoplankton; however, mean C:N (mass) values for POM in Pasqua ($6.0 + 1.7$ standard error) and Crooked lakes ($7.6 + 2.7$) encompassed the expected Redfield ratio for live phytoplankton (5.7). Further, this observation means that any bias in our calculations, which used C:N ratios slightly above Redfield values, resulted in an underestimate of total N demand by phytoplankton and, thereby, reinforces our principal conclusions.

We infer that our NAM approach does not underestimate the quantity of fixed N for several reasons. First, our POM-based estimates of N_2 fixation were higher than those derived from heterocytes, even though the latter method is highly correlated to independent estimates with both acetylene reduction and ^{15}N uptake techniques (Findlay et al., 1994; Higgins et al., 2017). Second, our rates of fixed N accumulation in the POM are like those measured independently (0.04 – 4.45 mg N m $^{-3}$ hr $^{-1}$) in these Qu'Appelle Lakes during 2017 using a ^{15}N -uptake assay (L. Boyer and H. Baulch, University of Saskatchewan, unpublished data). Third, estimates of N_2 fixation based on changes in $\delta^{15}N$ of the TDN pool provide values very similar to those derived from heterocyte determinations (Supporting Information Figure S4). Fourth, rate of hydrologic exchange (residence time) was not retained in any statistical model, making it unlikely that changes in N influx lead to an underestimation of N_2 fixation (Jankowski et al., 2012). Additionally, we note that rates of N_2 fixation in closed-basin Last Mountain Lake (Supporting Information Figure S8) were similar to those in other more highly flushed lakes. Fifth, in most lakes and years, retained fixed N is near zero and in 52% of samples the upper and lower bounds of NAM estimates include zero (Supporting Information Figure S7). Thus, while debate remains on the exact proportion of N demand required to be met by N_2 fixation to sustain P-limited growth of phytoplankton (Ferber et al., 2004), we suggest that in at least half of all 117 lake-years, diazotrophic supply represents a trivial source of N to natural pelagic assemblages in lakes where decadal averages of summer SRP concentration routinely exceed 100 μ g/L (Table 1) even during major blooms (Vogt et al., 2018).

5 | CONCLUSIONS

While fixed N was an occasionally important source of N to phytoplankton, there was limited evidence that N_2 fixation alleviated N deficits or initiated P limitation of phytoplankton growth in these highly P-rich ecosystems at catchment or decadal scales. While our conclusions may not apply to oligotrophic boreal lakes in undisturbed catchments, our conclusions should generalise

well to other lakes in continental landscapes with similar climatic, edaphic, and limnological characteristics—a region estimated to cover $>8 \times 10^6 \text{ km}^2$ (Finlay et al., 2015). For example, Qu'Appelle lakes exhibit similar snowmelt water sources (Pham et al., 2009), carbon fluxes (Finlay, Leavitt, et al., 2010), nitrogen biogeochemistry (Bogard et al., 2012), phosphorus dynamics (Donald et al., 2015) and land-use practises (Hall et al., 1999; Pham, Leavitt, McGowan, & Peres-Neto, 2008) as 100 other lakes within a 235,000 km^2 region of southern Saskatchewan. These basins have been shown to be good models for other large regional prairie lakes, including lakes Winnipeg and Manitoba (Bunting et al., 2016; Maheaux, Leavitt, & Jackson, 2016). Finally, similar to conclusions of Higgins et al. (2017), we note that low temporal coherence ($S = -0.04$) and high inter-annual variation in the magnitude of N supply by fixation (Figure 2) suggests that limnologists should be cautious in interpreting short annual or sub-decadal records as evidence of the role of N in supporting lake eutrophication.

ACKNOWLEDGMENTS

We thank members of the Limnology Laboratory for assistance with data collection since 1994. K. Hodder generated the map of our study sites. We also thank D.E. Schindler, S. Newell, M. McCarthy, J.T. Scott, A. Steinman, and several anonymous reviewers for reviews of an earlier draft of this paper. This work was supported by the NSERC Canada Discovery Grants program, Canada Research Chairs, Canada Foundation for Innovation, the Province of Saskatchewan, the University of Regina, and Queen's University Belfast. This paper is a contribution to the Qu'Appelle Long-term Ecological Research Program. We acknowledge that this research was conducted in Treaty 4 territory.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ORCID

Nicole M. Hayes  <https://orcid.org/0000-0002-5664-9939>

Gavin L. Simpson  <https://orcid.org/0000-0002-9084-8413>

Vanessa J. Swarbrick  <https://orcid.org/0000-0002-9323-6172>

Peter R. Leavitt  <https://orcid.org/0000-0001-9805-9307>

REFERENCES

- Abirhire, O., North, R. L., Hunter, K., Vandergucht, D. M., Sereda, J., & Hudson, J. J. (2015). Environmental factors influencing phytoplankton communities in Lake Diefenbaker, Saskatchewan, Canada. *Journal of Great Lakes Research*, 41, 118–128. <https://doi.org/10.1016/j.jglr.2015.07.002>
- Bennett, E. M., Carpenter, S. R., & Caraco, N. F. (2001). Human impact on erodable phosphorus and eutrophication: A global perspective. *BioScience*, 51, 227. [https://doi.org/10.1641/0006-3568\(2001\)051\[0227:hioepa\]2.0.co;2](https://doi.org/10.1641/0006-3568(2001)051[0227:hioepa]2.0.co;2)
- Bergman, B., Gallon, J. R., Rai, A. N., & Stal, L. J. (1997). N₂ fixation by non-heterocystous cyanobacteria. *FEMS Microbiology Reviews*, 19, 139–185. [https://doi.org/10.1016/s0168-6445\(96\)00028-9](https://doi.org/10.1016/s0168-6445(96)00028-9)
- Beversdorf, L. J., Miller, T. R., & McMahon, K. D. (2013). The role of nitrogen fixation in cyanobacterial bloom toxicity in a temperate, eutrophic lake. *PLoS One*, 8, e56103. <https://doi.org/10.1371/journal.pone.0056103>
- Bodirsky, B. L., Popp, A., Lotze-Campen, H., Dietrich, J. P., Rolinski, S., Weindl, I., ... Stevanovic, M. (2014). Reactive nitrogen requirements to feed the world in 2050 and potential to mitigate nitrogen pollution. *Nature Communications*, 5, 1–7.
- Bogard, M. J., Donald, D. B., Finlay, K., & Leavitt, P. R. (2012). Distribution and regulation of urea in lakes of central North America. *Freshwater Biology*, 57, 1277–1292. <https://doi.org/10.1111/j.1365-2427.2012.02775.x>
- Bruesewitz, D. A., Hamilton, D. P., & Schipper, L. A. (2011). Denitrification potential in lake sediment increases across a gradient of catchment agriculture. *Ecosystems*, 14, 341–352. <https://doi.org/10.1007/s10021-011-9413-2>
- Bunting, L., Leavitt, P. R., Gibson, C. E., McGee, E. J., & Hall, V. A. (2007). Degradation of water quality in Lough Neagh, Northern Ireland, by diffuse nitrogen flux from a phosphorus-rich catchment. *Limnology & Oceanography*, 52, 354–369. <https://doi.org/10.4319/lo.2007.52.1.0354>
- Bunting, L., Leavitt, P. R., Simpson, G. L., Wissel, B., Laird, K. R., Cumming, B. F., ... Engstrom, D. (2016). Increased variability and sudden ecosystem state change in Lake Winnipeg, Canada, caused by 20th century agriculture. *Limnology and Oceanography*, 61, 2090–2107. <https://doi.org/10.1002/lno.10355>
- Carpenter, S. R. (2005). Eutrophication of aquatic ecosystems: Bistability and soil phosphorus. *Proceedings of the National Academy of Sciences of the United States of America*, 102, 10002–10005.
- Carpenter, S. R., Caraco, N. F., Correll, D. L., Howarth, R. W., Sharpley, A. N., & Smith, V. H. (1998). Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecological Applications*, 8, 559–568. [https://doi.org/10.1890/1051-0761\(1998\)008\[0559:nposww\]2.0.co;2](https://doi.org/10.1890/1051-0761(1998)008[0559:nposww]2.0.co;2)
- Carpenter, S. R., Cole, J. J., Hodgson, J. R., Kitchell, J. F., Pace, M. L., Bade, D. L., ... Schindler, D. E. (2001). Trophic cascades, nutrients, and lake productivity: Whole-lake experiments. *Ecological Monographs*, 71, 163–186. [https://doi.org/10.1890/0012-9615\(2001\)071\[0163:tcnalp\]2.0.co;2](https://doi.org/10.1890/0012-9615(2001)071[0163:tcnalp]2.0.co;2)
- David, M. B., Wall, L. G., Royer, T. V., & Tank, J. L. (2006). Denitrification and the nitrogen budget of a reservoir in an agricultural landscape. *Ecological Applications*, 16, 2177–2190. [https://doi.org/10.1890/1051-0761\(2006\)016\[2177:datnbo\]2.0.co;2](https://doi.org/10.1890/1051-0761(2006)016[2177:datnbo]2.0.co;2)
- Deininger, A., Faithfull, C. L., & Bergström, A. K. (2017). Phytoplankton response to whole lake inorganic N fertilization along a gradient in dissolved organic carbon. *Ecology*, 98, 982–994. <https://doi.org/10.1002/ecy.1758>
- Dillon, P. J., & Rigler, F. (1974). The phosphorus-chlorophyll in lakes. *Limnology and Oceanography*, 19, 767–773. <https://doi.org/10.4319/lo.1974.19.5.0767>
- Dolman, A. M., Rücker, J., Pick, F. R., Fastner, J., Rohrlack, T., Mischke, U., & Wiedner, C. (2012). Cyanobacteria and cyanotoxins: The influence of nitrogen versus phosphorus. *PLoS One*, 7, e38757. <https://doi.org/10.1371/journal.pone.0038757>
- Donald, D. B., Bogard, M. J., Finlay, K., Bunting, L., & Leavitt, P. R. (2013). Phytoplankton-specific response to enrichment of phosphorus-rich surface waters with ammonium, nitrate, and urea. *PLoS One*, 8, e53277. <https://doi.org/10.1371/journal.pone.0053277>
- Donald, D. B., Bogard, M. J., Finlay, K., & Leavitt, P. R. (2011). Comparative effects of urea, ammonium, and nitrate on phytoplankton abundance, community composition, and toxicity in hypereutrophic freshwaters. *Limnology and Oceanography*, 56, 2161–2175. <https://doi.org/10.4319/lo.2011.56.6.2161>

- Donald, D. B., Parker, B. R., Davies, J.-M., & Leavitt, P. R. (2015). Nutrient sequestration in the Lake Winnipeg watershed. *Journal of Great Lakes Research*, 41, 630–642. <https://doi.org/10.1016/j.jglr.2015.03.007>
- Dröscher, I., Patoine, A., Finlay, K., & Leavitt, P. R. (2009). Climate control of the spring clear-water phase through the transfer of energy and mass to lakes. *Limnology and Oceanography*, 54, 2469–2480. https://doi.org/10.4319/lo.2009.54.6_part_2.2469
- Elliott, J. A. (2010). The seasonal sensitivity of Cyanobacteria and other phytoplankton to changes in flushing rate and water temperature. *Global Change Biology*, 16, 864–876. <https://doi.org/10.1111/j.1365-2486.2009.01998.x>
- Elser, J. J., Sterner, R. W., Galford, A. E., Chrzanowski, T. H., Findlay, D. L., Mills, K. H., ... Schindler, D. W. (2000). Pelagic C:N:P stoichiometry in a eutrophic lake: Responses to a whole-lake food-web manipulation. *Ecosystems*, 3, 293–307. <https://doi.org/10.1007/s100210000027>
- Ferber, L. R., Levine, S. N., Lini, A., & Livingston, G. P. (2004). Do cyanobacteria dominate in eutrophic lakes because they fix atmospheric nitrogen? *Freshwater Biology*, 49, 690–708. <https://doi.org/10.1111/j.1365-2427.2004.01218.x>
- Findlay, D. L., Hecky, R. E., Hendzel, L. L., Stainton, M. P., & Regehr, G. W. (1994). Relationship between N₂-fixation and heterocyst abundance and its relevance to the nitrogen budget of lake 227. *Canadian Journal of Fisheries and Aquatic Sciences*, 51, 2254–2266. <https://doi.org/10.1139/f94-229>
- Finlay, K., Leavitt, P. R., Patoine, A., & Wissel, B. (2010). Magnitudes and controls of organic and inorganic carbon flux through a chain of hard-water lakes on the northern Great Plains. *Limnology and Oceanography*, 55, 1551–1564. <https://doi.org/10.4319/lo.2010.55.4.1551>
- Finlay, K., Leavitt, P. R., Wissel, B., & Prairie, Y. T. (2009). Regulation of spatial and temporal variability of carbon flux in six hard-water lakes of the northern Great Plains. *Limnology and Oceanography*, 54, 2553–2564. https://doi.org/10.4319/lo.2009.54.6_part_2.2553
- Finlay, K., Patoine, A., Donald, D. B., Bogard, M. J., & Leavitt, P. R. (2010). Experimental evidence that pollution with urea can degrade water quality in phosphorus-rich lakes of the northern Great Plains. *Limnology and Oceanography*, 55, 1213–1230. <https://doi.org/10.4319/lo.2010.55.3.1213>
- Finlay, K., Vogt, R. J., Bogard, M. J., Wissel, B., Tutolo, B. M., Simpson, G. L., & Leavitt, P. R. (2015). Decrease in CO₂ efflux from northern hardwater lakes with increasing atmospheric warming. *Nature*, 519, 215–218. <https://doi.org/10.1038/nature14172>
- Flores, E., & Herrero, A. (2005). Nitrogen assimilation and nitrogen control in cyanobacteria. *Biochemical Society Transactions*, 33, 164–167. <https://doi.org/10.1042/bst0330164>
- Fowler, D., Coyle, M., Skiba, U., Sutton, M. A., Cape, J. N., Reis, S., ... Voss, M. (2013). The global nitrogen cycle in the twenty-first century. *Philosophical Transactions of the Royal Society of London. Series B, Biological sciences*, 368, 1–13.
- Fulweiler, R. W., Heiss, E. M., Rogener, M. K., Newell, S. E., LeClerc, G. R., Kortebein, S. M., & Wilhelm, S. W. (2015). Examining the impact of acetylene on N-fixation and the active sediment microbial community. *Frontiers in Microbiology*, 6, 1–9.
- Glibert, P. M., & Bronk, D. A. (1994). Release of dissolved organic nitrogen by marine diazotrophic cyanobacteria, *Trichodesmium* spp. *Applied and Environmental Microbiology*, 60, 3996–4000.
- Glibert, P. M., Maranger, R., Sobota, D. J., & Bouwman, L. (2014). The Haber Bosch – harmful algal bloom (HB – HAB) link. *Environmental Research Letters*, 9, 105001. <https://doi.org/10.1088/1748-9326/9/10/105001>
- Hall, R. I., Leavitt, P. R., Quinlan, R., Dixit, A. S., & Smol, J. P. (1999). Effects of agriculture, urbanization, and climate on water quality in the northern Great Plains. *Limnology and Oceanography*, 44, 739–756. https://doi.org/10.4319/lo.1999.44.3_part_2.0739
- Hammer, U. T. (1971). Limnological studies of the lakes and streams of the upper Qu'Appelle River system, Saskatchewan, Canada I. Chemical and physical aspects of the lakes and drainage system. *Hydrobiologia*, 37, 473–507. <https://doi.org/10.1007/bf00018815>
- Harke, M. J., Steffen, M. M., Gobler, C. J., Otten, T. G., Wilhelm, S. W., Wood, S. A., & Paerl, H. W. (2016). A review of the global ecology, genomics, and biogeography of the toxic cyanobacterium, *Microcystis* spp. *Harmful Algae*, 54, 4–20. <https://doi.org/10.1016/j.hal.2015.12.007>
- Hendzel, L. L., Hecky, R. E., & Findlay, D. L. (1994). Recent changes of N₂-fixation in Lake 227 in response to reduction of the N:P loading ratio. *Canadian Journal of Fisheries and Aquatic Sciences*, 51, 2247–2253. <https://doi.org/10.1139/f94-228>
- Herrero, A., Stavans, J., & Flores, E. (2016). The multicellular nature of filamentous heterocyst-forming cyanobacteria. *FEMS Microbiology Reviews*, 40, 831–854. <https://doi.org/10.1093/femsre/fuw029>
- Higgins, S. N., Paterson, M. J., Hecky, R. E., Schindler, D. W., Venkiteswaran, J. J., & Findlay, D. L. (2017). Biological nitrogen fixation prevents the response of a eutrophic lake to reduced loading of nitrogen: Evidence from a 46-year whole-lake experiment. *Ecosystems*, 21, 1088–1100.
- Howarth, R. W., Marino, R., Cole, J. J., Lane, J., & Howarth, W. (1988). Nitrogen fixation in freshwater, estuarine, and marine ecosystems. 2. Biogeochemical controls. *Limnology and Oceanography*, 33, 688–701.
- Howarth, R. W., Marino, R., Lane, J., & Cole, J. J. (1988). Nitrogen fixation in freshwater, estuarine, and marine ecosystems. 1. Rates and importance. *Limnology and Oceanography*, 33, 669–687.
- Jankowski, K., Schindler, D. E., & Holtgrieve, G. W. (2012). Assessing nonpoint-source nitrogen loading and nitrogen fixation in lakes using $\delta^{15}\text{N}$ and nutrient stoichiometry. *Limnology and Oceanography*, 57, 671–683. <https://doi.org/10.4319/lo.2012.57.3.0671>
- Jeppesen, E., Sondergaard, M., Jensen, J. P., Havens, K. E., Anneville, O., Carvalho, L., ... Winder, M. (2005). Lake responses to reduced nutrient loading – An analysis of contemporary long-term data from 35 case studies. *Freshwater Biology*, 50, 1747–1771. <https://doi.org/10.1111/j.1365-2427.2005.01415.x>
- Jöhnk, K. D., Huisman, J., Sharples, J., Sommeijer, B., Visser, P. M., & Stroom, J. M. (2008). Summer heatwaves promote blooms of harmful cyanobacteria. *Global Change Biology*, 14, 495–512. <https://doi.org/10.1111/j.1365-2486.2007.01510.x>
- Karl, D., Letelier, R., Tupas, L., Dore, J., Christian, J., & Hebel, D. (1997). The role of nitrogen fixation in biogeochemical cycling in the subtropical North Pacific Ocean. *Nature*, 388, 533–538. <https://doi.org/10.1038/41474>
- Kilinc, S., & Moss, B. (2002). Whitemere, a lake that defies some conventions about nutrients. *Freshwater Biology*, 47, 207–218. <https://doi.org/10.1046/j.1365-2427.2002.00797.x>
- Klassen, R. W. (1989). Quaternary geology of southern Canadian Interior Plains. In R. J. Fulton (Ed.), *Quaternary geology of Canada and Greenland* (pp. 138–174). Ottawa, Canada: Geological Survey of Canada.
- Lathrop, R. C. (2007). Perspectives on the eutrophication of the Yahara lakes. *Lake and Reservoir Management*, 23, 345–365. <https://doi.org/10.1080/07438140709354023>
- Leavitt, P. R., Brock, C. S., Ebel, C., & Patoine, A. (2006). Landscape-scale effects of urban nitrogen on a chain of freshwater lakes in central North America. *Limnology and Oceanography*, 51, 2262–2277. <https://doi.org/10.4319/lo.2006.51.5.2262>
- Leavitt, P. R., Fritz, S. C., Anderson, N. J., Baker, P. A., Blenckner, T., Bunting, L., ... Werne, J. (2009). Paleolimnological evidence of the effects on lakes of energy and mass transfer from climate and humans. *Limnology & Oceanography*, 54, 2330–2348. https://doi.org/10.4319/lo.2009.54.6_part_2.2330
- Leavitt, P. R., & Hodgson, D. A. (2001). Sedimentary pigments. In J. P. Smol, H. J. B. Birks, W. M. Last, R. S. Bradley, & K. Alverson (Eds.), *Tracking environmental change using lake sediments* (pp. 295–325). Dordrecht, Netherlands: Springer Netherlands.
- Levine, S. N., & Schindler, D. W. (1999). Influence of nitrogen to phosphorus supply ratios and physicochemical conditions on cyanobacteria

- and phytoplankton species composition in the Experimental Lakes Area, Canada. *Canadian Journal of Fisheries and Aquatic Sciences*, 56, 451–466. <https://doi.org/10.1139/f98-183>
- MacIntyre, H. L., Kana, T. M., Anning, T., & Geider, R. J. (2002). Photoacclimation of photosynthesis irradiance response curves and photosynthetic pigments in microalgae and cyanobacteria. *Journal of Phycology*, 38, 17–38. <https://doi.org/10.1046/j.1529-8817.2002.00094.x>
- Maheux, H., Leavitt, P. R., & Jackson, L. J. (2016). Asynchronous onset of eutrophication among shallow prairie lakes of the northern Great Plains, Alberta, Canada. *Global Change Biology*, 22, 271–283. <https://doi.org/10.1111/gcb.13076>
- Marino, R., Howarth, R. W., Chan, F., Cole, J. J., & Likens, G. E. (2003). Sulfate inhibition of molybdenum-dependent nitrogen fixation by planktonic cyanobacteria under seawater conditions: A non-reversible effect. *Hydrobiologia*, 500, 277–293. <https://doi.org/10.1023/a:1024641904568>
- Marra, G., & Wood, S. N. (2011). Practical variable selection for generalized additive models. *Computational Statistics and Data Analysis*, 55, 2372–2387. <https://doi.org/10.1016/j.csda.2011.02.004>
- McCrackin, M. L., Jones, H. P., Jones, P. C., & Moreno-Mateos, D. (2016). Recovery of lakes and coastal marine ecosystems from eutrophication: A global meta-analysis. *Limnology and Oceanography*, 62, 507–518.
- McGowan, S., Leavitt, P. R., & Hall, R. I. (2005). A whole-lake experiment to determine the effects of winter droughts on shallow lakes. *Ecosystems*, 8, 694–708. <https://doi.org/10.1007/s10021-003-0152-x>
- Moe, S. J., Stelzer, R. S., Forman, M. R., Harpole, W. S., Daufresne, T., & Yoshida, T. (2005). Recent advances in ecological stoichiometry: Insights for population and community ecology. *Oikos*, 109, 29–39. <https://doi.org/10.1111/j.0030-1299.2005.14056.x>
- Moss, B., Jeppesen, E., Søndergaard, M., Lauridsen, T. L., & Liu, Z. (2013). Nitrogen, macrophytes, shallow lakes and nutrient limitation: Resolution of a current controversy? *Hydrobiologia*, 710, 3–21. <https://doi.org/10.1007/s10750-012-1033-0>
- Mugidde, R., Hecky, R. E., Hendzel, L. L., & Taylor, W. D. (2003). Pelagic nitrogen fixation in Lake Victoria (East Africa). *Journal of Great Lakes Research*, 29, 76–88. [https://doi.org/10.1016/s0380-1330\(03\)70540-1](https://doi.org/10.1016/s0380-1330(03)70540-1)
- Pacheco, F. S., Roland, F., & Downing, J. A. (2014). Eutrophication reverses whole-lake carbon budgets. *Inland Waters*, 4, 41–48. <https://doi.org/10.5268/iw>
- Paerl, H. W., & Scott, J. T. (2010). Throwing fuel on the fire: Synergistic effects of excessive nitrogen inputs and global warming on harmful algal blooms. *Environmental Science & Technology*, 44, 7756–7758. <https://doi.org/10.1021/es102665e>
- Paerl, H. W., Scott, J. T., McCarthy, M. J., Newell, S. E., Gardner, W., Havens, K. E., ... Wurtsbaugh, W. A. (2016). It takes two to tango: When and where dual nutrient (N & P) reductions are needed to protect lakes and downstream ecosystems. *Environmental Science & Technology*, 50, 10805–10813. <https://doi.org/10.1021/acs.est.6b02575>
- Paterson, M. J., Schindler, D. W., Hecky, R. E., Findlay, D. L., & Rondeau, K. J. (2011). Comment: Lake 227 shows clearly that controlling inputs of nitrogen will not reduce or prevent eutrophication of lakes. *Limnology and Oceanography*, 56, 1545–1547. <https://doi.org/10.4319/lo.2011.56.4.1545>
- Patoine, A., Graham, M. D., & Leavitt, P. R. (2006). Spatial variation of nitrogen fixation in lakes of the northern Great Plains. *Limnology and Oceanography*, 51, 1665–1677. <https://doi.org/10.4319/lo.2006.51.4.1665>
- Patoine, A., & Leavitt, P. R. (2008). Landscape analysis of the role of N₂ fixation in satisfying algal demand for nitrogen in eutrophic lakes. *Verhandlungen Internationale Vereinigung für Theoretische und Angewandte Limnologie*, 30, 366–370.
- Peterson, B. J., & Fry, B. (1987). Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics*, 18, 293–320. <https://doi.org/10.1146/annurev.es.18.110187.001453>
- Pham, S. V., Leavitt, P. R., McGowan, S., & Peres-Neto, P. (2008). Spatial variability of climate and land-use effects on lakes of the northern Great Plains. *Limnology and Oceanography*, 53, 728–742. <https://doi.org/10.4319/lo.2008.53.2.0728>
- Pham, S. V., Leavitt, P. R., McGowan, S., Wissel, B., & Wassenaar, L. I. (2009). Spatial and temporal variability of prairie lake hydrology as revealed using stable isotopes of hydrogen and oxygen. *Limnology and Oceanography*, 54, 101–118. <https://doi.org/10.4319/lo.2009.54.1.0101>
- Quiñones-Rivera, Z. J., Finlay, K., Vogt, R. J., Leavitt, P. R., & Wissel, B. (2015). Hydrologic, metabolic and chemical regulation of water-column metabolism and atmospheric CO₂ exchange in a large continental reservoir during spring and summer. *Journal of Great Lakes Research*, 41, 144–154. <https://doi.org/10.1016/j.jglr.2015.05.005>
- R Core Team (2017). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from <http://www.R-project.org/>
- Rice, E. W., Baird, R. B., Eaton, A. D., & Clesceri, L. S. (2012). *Standard methods for the examination of water and wastewater* (22nd ed.). Washington, DC: American Public Health Association, American Water Works Association, Water Environment Federation.
- Sala, O. E., Jackson, R. B., Mooney, H. A., & Howarth, R. W. (Eds.) (2000). *Methods in ecosystem science*. New York, NY: Springer-Verlag.
- Savage, C., Leavitt, P. R., & Elmgren, R. (2004). Distribution and retention of sewage nitrogen in surface sediments of a coastal bay. *Limnology and Oceanography*, 49, 1503–1511. <https://doi.org/10.4319/lo.2004.49.5.1503>
- Schindler, D. W. (1977). Evolution of phosphorus limitation in lakes. *Science*, 195, 260–262. <https://doi.org/10.1126/science.195.4275.260>
- Schindler, D. W. (2006). Recent advances in the understanding and management of eutrophication. *Limnology and Oceanography*, 51, 356–363. https://doi.org/10.4319/lo.2006.51.1_part_2.0356
- Schindler, D. W., Carpenter, S. R., Chapra, S. C., Hecky, R. E., & Orihel, D. M. (2016). Reducing phosphorus to curb lake eutrophication is a success. *Environmental Science and Technology*, 50, 8923–8929. <https://doi.org/10.1021/acs.est.6b02204>
- Schindler, D. E., Carpenter, S. R., Cole, J. J., Kitchell, J. F., & Pace, M. L. (1997). Influence of food web structure on carbon exchange between lakes and the atmosphere. *Science*, 277, 248–251. <https://doi.org/10.1126/science.277.5323.248>
- Schindler, D. W., Hecky, R. E., Findlay, D. L., Stainton, M. P., Parker, B. R., Paterson, M. J., ... Kasian, S. E. M. (2008). Eutrophication of lakes cannot be controlled by reducing nitrogen input: Results of a 37-year whole-ecosystem experiment. *Proceedings of the National Academy of Sciences of the United States of America*, 105, 11254–11258. <https://doi.org/10.1073/pnas.0805108105>
- Scott, J. T., & Grantz, E. M. (2013). N₂ fixation exceeds internal nitrogen loading as a phytoplankton nutrient source in perpetually nitrogen-limited reservoirs. *Freshwater Science*, 32, 849–861. <https://doi.org/10.1899/12-190.1>
- Scott, J. T., & McCarthy, M. J. (2010). Nitrogen fixation may not balance the nitrogen pool in lakes over timescales relevant to eutrophication management. *Limnology and Oceanography*, 55, 1265–1270. <https://doi.org/10.4319/lo.2010.55.3.1265>
- Scott, J. T., & McCarthy, M. J. (2011). Response to Comment: Nitrogen fixation has not offset declines in the Lake 227 nitrogen pool and shows that nitrogen control deserves consideration in aquatic ecosystems. *Limnology and Oceanography*, 56, 1548–1550. <https://doi.org/10.4319/lo.2011.56.4.1548>
- Scott, J. T., Stanley, J. K., Doyle, R. D., Forbes, M. G., & Brooks, B. W. (2009). River-reservoir transition zones are nitrogen fixation hot

- spots regardless of ecosystem trophic state. *Hydrobiologia*, 625, 61–68. <https://doi.org/10.1007/s10750-008-9696-2>
- Seitzinger, S., Harrison, J., Bohlke, J., Bouwman, A., Lowrance, R., Peterson, B., ... Van Drecht, G. (2006). Denitrification across landscapes and waterscapes: A synthesis. *Ecological Applications*, 16, 2064–2090. [https://doi.org/10.1890/1051-0761\(2006\)016\[2064:dalawaj\]2.0.co;2](https://doi.org/10.1890/1051-0761(2006)016[2064:dalawaj]2.0.co;2)
- Smith, V. H. (1983). Low nitrogen to phosphorus ratios favor dominance by blue-green algae in lake phytoplankton. *Science*, 221, 669–671. <https://doi.org/10.1126/science.221.4611.669>
- Smith, V. H., & Schindler, D. W. (2009). Eutrophication science: Where do we go from here? *Trends in Ecology & Evolution*, 24, 201–207. <https://doi.org/10.1016/j.tree.2008.11.009>
- Soranno, P. A., Webster, K. E., Riera, J. L., Kratz, T. K., Baron, J. S., Bukaveckas, P. A., ... Leavitt, P. R. (1999). Spatial variation among lakes within landscapes: Ecological organization along lake chains. *Ecosystems*, 2, 395–410. <https://doi.org/10.1007/s100219900089>
- Stainton, M. J., Capel, M. J., & Armstrong, A. J. (1977). *The chemical analysis of freshwater* (2nd ed.). Can. Fish. Mar. Serv. Misc. Spec. Publ., 25.
- Stoddard, J. L., Van Sickle, J., Herlihy, A. T., Brahmey, J., Paulsen, S., Peck, D. V., ... Pollard, A. I. (2016). Continental-scale increase in lake and stream phosphorus: Are oligotrophic systems disappearing in the United States? *Environmental Science and Technology*, 50, 3409–3415. <https://doi.org/10.1021/acs.est.5b05950>
- Torrey, M. S., & Lee, G. F. (1976). Nitrogen fixation in Lake Mendota, Madison, Wisconsin. *Limnology and Oceanography*, 21, 365–378. <https://doi.org/10.4319/lo.1976.21.3.0365>
- Utermöhl, H. (1958). Zur vervollkommung der quantitative phytoplankton metodik. *Mitteilungen Internationale Vereinigung für Theoretische und Angwandte Limnologie*, 9, 1–38.
- Vitousek, P. M., Menge, D. N. L., Reed, S. C., & Cleveland, C. C. (2013). Biological nitrogen fixation: Rates, patterns and ecological controls in terrestrial ecosystems. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 368, 20130119. <https://doi.org/10.1098/rstb.2013.0119>
- Vogt, R. J., Rusak, J. A., Patoine, A., & Leavitt, P. R. (2011). Differential effects of energy and mass influx on the landscape synchrony of lake ecosystems. *Ecology*, 92, 1104–1114. <https://doi.org/10.1890/10-1846.1>
- Vogt, R. J., Sharma, S., & Leavitt, P. R. (2018). Direct and interactive effects of climate, meteorology, river hydrology, and lake characteristics on water quality in productive lakes of the Canadian Prairies. *Canadian Journal of Fisheries and Aquatic Sciences*, 75, 47–59. <https://doi.org/10.1139/cjfas-2016-0520>
- Vrede, T., Ballantyne, A., Mille-Lindblom, C., Algesten, G., Gudas, C., Lindahl, S., & Brunberg, A. K. (2009). Effects of N: P loading ratios on phytoplankton community composition, primary production and N fixation in a eutrophic lake. *Freshwater Biology*, 54, 331–344. <https://doi.org/10.1111/j.1365-2427.2008.02118.x>
- Wood, S. N. (2011). Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. *Journal of the Royal Statistical Society: Series B (Statistical Methodology)*, 73, 3–36. <https://doi.org/10.1111/j.1467-9868.2010.00749.x>
- Wood, S. N., Pya, N., & Säfken, B. (2016). Smoothing parameter and model selection for general smooth models. *Journal of the American Statistical Association*, 111, 1548–1563. <https://doi.org/10.1080/01621459.2016.1180986>
- Zhang, X., Sigman, D. M., Morel, F. M. M., & Kraepiel, A. M. L. (2014). Nitrogen isotope fractionation by alternative nitrogenases and past ocean anoxia. *Proceedings of the National Academy of Sciences of the United States of America*, 111, 4782–4787. <https://doi.org/10.1073/pnas.1402976111>

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Hayes NM, Patoine A, Haig HA, et al. Spatial and temporal variation in nitrogen fixation and its importance to phytoplankton in phosphorus-rich lakes. *Freshw Biol.* 2018;00:1–15. <https://doi.org/10.1111/fwb.13214>