Phosphorus-only fertilization rapidly initiates large nitrogen-fixing cyanobacteria blooms in two oligotrophic lakes

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Abstract

Two small, oligotrophic lakes at the IISD-Experimental Lakes Area in northwestern Ontario, Canada were fertilized weekly with only phosphorus (P) in the summer and early fall of 2019. The P fertilization rates were high enough (13.3 µg l⁻¹ added weekly) to produce dense, month-long blooms of N₂-fixing Dolichospermum species in both lakes within 9–12 weeks after fertilization began, turning them visibly green without the addition of nitrogen. P-only fertilization increased average seasonal chlorophyll a concentrations and cyanobacteria biomass well above the pre-fertilization levels of 2017 and 2018. Nitrogen (N) content in the epilimnion of thermally stratified Lake 304 and the water column of shallow Lake 303 doubled and P storage in the water column temporarily increased during the blooms. These whole-lake fertilization experiments demonstrate that large cyanobacteria blooms can develop rapidly under high P loading without anthropogenic N inputs, suggesting that aggressive N control programs are unlikely to prevent bloom formation and that P controls should remain the cornerstone for cyanobacteria management.

1. Introduction

Filamentous and colonial cyanobacteria blooms, a hallmark characteristic of nutrient-enriched (eutrophic) waters, are a major management concern because they can produce liver and neuro-toxins as well as taste and odour compounds that alter the quality of drinking water supplies (Watson et al 2008). Hence, bloom prevention remains a high management concern, especially as bloom incidence has been increasing in eutrophic as well as low nutrient (oligotrophic) lakes (Paerl and Huisman 2009, Winter et al 2011, Scavia et al 2014).

Cyanobacteria responses to additions of nitrogen (N) and phosphorus (P), two key nutrients, to P-limited lakes, and nutrient controls to eutrophic lakes have been clouded by the small number of P-only whole-lake fertilization experiments in non-arctic regions where nuisance pelagic cyanobacteria are capable of forming blooms. The gold standard for understanding how nutrients affect lake ecosystems is controlled whole-lake fertilization even though such experiments may lack spatial replication. Smaller scale studies such as closed bottle assays and mesocosms without sediments, while easier to replicate, exclude important processes such as sediment-water processes, gas exchange and food web interactions. Moreover, they are of relatively short duration to avoid resource limitation (bottles) and wall effects (mesocosms). Since many assumptions must be
made in order to extrapolate smaller scale results to whole ecosystems, conclusions can be problematic (Carpenter 1996, Schindler 2012). However, bottle and mesocosm methods are suited to the study of certain short-term phenomena.

Few, well controlled, whole-lake fertilization experiments that examine the impacts of fertilization on development of pelagic filamentous and colonial cyanobacteria blooms in natural oligotrophic systems with clearly documented baseline (before fertilization) conditions and carefully controlled additions are available globally. In previous whole-lake experiments at the International Institute for Sustainable Development (IISD)-Experimental Lakes Area (ELA) in the boreal forest in northwestern Ontario, several P-limited, circum-neutral oligotrophic lakes and isolated basins were fertilized with N or N and P, and in some cases with carbon, to understand how lakes respond to nutrient additions. For example, blooms of N₂ fixing cyanobacteria developed in the north east basin of Lake 226 which was fertilized with P, N and carbon (C) (Findlay and Kasian 1987) implicating the role of P in bloom formation in this P-limited lake but did not form in a basin to which N and C had been added. What is not known would be the magnitude of the bloom and how fast blooms would have developed if the basin had been fertilized with only P.

Two ELA lakes have been fertilized with only P: Lake 261 between 1973 and 1976 and Lake 227 after 1990 (Lewis and Wurtsbaugh 2008, Schindler et al 2008, Schindler 2012). The P-only fertilization of Lake 261 did not result in cyanobacteria blooms in the water column during four years of fertilization and the annual ice-free total P in the top 2 m of the lake was not very high, ranging from 18 to 23 µg l⁻¹. The absence of cyanobacteria blooms could suggest that the P fertilization rate was too low to create eutrophic conditions necessary for bloom formation. The relatively small increase in total P may be related to its shallow nature (mean depth 2.9 m). With 49% of the sediment area situated at depths less than 2 m there might have been significant competition for P from benthic and epiphytic communities.

Lake 227 is the site of an ongoing 50 year experiment at a constant P fertilization rate, receiving N as nitrate for the first 20 years and only P for the last 30 years making it the only whole-lake experiment that has examined the long-term effects of anthropogenic N removal and the only whole-lake P-only fertilization experiment to date which has successfully generated eutrophic conditions and cyanobacteria blooms outside of the arctic (Schindler et al 2008, Paterson et al 2011, Higgins et al 2018). This experiment has not been replicated at other ELA lakes but repeated annual observations for 30 years during the P-only phase lend confidence to its results. Since the P-only phase followed 20 years of N fertilization, it is not known how rapidly blooms would have developed in the absence of N fertilization.

Several P-only whole-lake fertilization experiments have been conducted outside ELA, mostly in far northern regions (Lundgren 1978a, 1978b, McCoy 1983, Jansson 1984, Björk-Ramberg and Ånell 1985, Welch et al 1989, Bergman and Welch 1990, Jansson et al 2001) where pelagic non-pico forms of cyanobacteria are naturally very rare even in eutrophic waters inferring that they cannot grow there (Schindler et al 1974, Schlesinger et al 1981, Vincent 2000, Rautio et al 2011, Vincent and Quesada 2012). While microcystins have been detected in Greenland freshwaters (Trout-Haney et al 2016), phytoplankton were not enumerated in that study and it is more likely that benthic cyanobacteria, common to arctic waters, were the source of the toxins (benthic species can produce microcystins (Izaguirre et al 2007, Wood et al 2010)). While these arctic studies contribute to our understanding of how P fertilization of oligotrophic lakes affects productivity, nutrient cycling and food web interactions, their results cannot be used to understand the responses of pelagic, non-pico forms of cyanobacteria.

Outside the arctic, three lakes near Schefferville, Quebec in Canada were fertilized with P but spring concentrations post-fertilization did not exceed 10 µg l⁻¹, remaining in the oligotrophic range (Smith et al 1984). Two other P-only fertilization studies were conducted in sites that were atypical of oligotrophic lakes globally: an artificial pond that was eutrophic before fertilization (Li et al 2017) and Lake Lundetjenn in southern Norway which was acidic with pH 5 (Donali et al 2005).

The collective results of these whole-lake fertilizations of oligotrophic lakes have been inconclusive about the respective roles of N and P in generating cyanobacteria blooms because only one P-only experiment resulted in P levels high enough outside the arctic to generate cyanobacteria blooms, Lake 227. In spite of this study’s longevity and high quality, 20 years of N additions prior to the P-only phase and the overall lack of replication at other sites has inhibited resolution of the debate about whether cyanobacteria management programs should remain focused on P or whether N control programs should be used along side P control programs to prevent or at least partially mitigate cyanobacteria blooms (Glibert et al 2014, Lewis and Wurtsbaugh 2008, Lewis et al 2011, Pael et al 2011, Schindler 2012, Schindler et al 2016, Higgins et al 2018, Molot et al 2021). More strictly P-only whole-lake fertilization experiments with addition rates high enough to promote eutrophic conditions and cyanobacteria blooms in regions outside of the arctic and in different types of P-limited oligotrophic lakes are needed, e.g. thermally stratified and polymeric (shallow and well mixed), to determine whether a P-fertilized lake can rapidly develop large nuisance cyanobacteria.
blooms and whether large increases in chlorophyll $a$ occur without adding anthropogenic N. To partially address this information gap, we fertilized two small, P-limited, circum-neutral oligotrophic lakes at ELA with P under current climatic conditions. Lake 303 is polymictic (mean depth 1.5 m) while Lake 304 is deeper (mean depth 3.2 m) and thermally stratifies, thus providing an opportunity to study the cyanobacteria responses to P fertilization in lakes with different thermal regimes and proportions of shallow areas.

2. Methods

2.1. Study sites

The ELA (www.iisd.org/ela/) is located on the Precambrian Shield of northwestern Ontario approximately 40 km east of Lake of the Woods. The Lake 303 and Lake 304 watersheds consist of coniferous forests with thin soils, rock outcroppings and small wetlands and are underlain by granitic bedrock. The lakes, located adjacent to each other, are headwater lakes with minimal groundwater inputs. Lake 303 is polymictic, with mean and maximum depths of 1.5 and 2.5 m, surface area 9.93 ha, terrestrial catchment area 44.2 ha and water renewal time 1.1 years (Newbury and Beaty 1980). Rainbow trout were introduced in 1969 and it was fertilized in 1975 and 1984 with P and N (Levine and Schindler 1989). Lake 304 thermally stratifies with mean and maximum depths of 3.2 and 6.7 m, surface area 3.62 ha, terrestrial catchment area 23 ha and water renewal time 1.8 years (Newbury and Beaty 1980). Rainbow trout were introduced in 1969 and the lake was fertilized in 1972 with P, N and C, in 1974 with N and C, and in 1976 with P and N. Rainbow trout did not survive in either lake. These fertilization treatments occurred long enough ago (35–47 years ago) and were repeated only a few times (two and three years in Lakes 303 and 304, respectively) that it is safe to assume the nutrients added 35+ years ago (i.e. ‘legacy’ N) were not available to phytoplankton in 2019, i.e. they are sequestered in sediments or have been lost downstream or to the atmosphere, and that the earlier fertilization experiments have not affected the current one (Paterson et al 2011, Higgins et al 2018). Lake 303 is shallower with 68% of its sediment area at depths less than 2 m, compared to 30% in Lake 304.

2.2. Fertilization and sampling protocols

Lakes 303 and 304 were fertilized weekly with equal amounts of P per unit total lake volume from 6 June until 17 October in 2019. Lake 303 was fertilized with 2 kg P per week (an addition of 13.3 $\mu$g l$^{-1}$), double the weekly load in the earlier fertilization experiments, while Lake 304 was fertilized with 1.54 kg P per week (13.4 $\mu$g l$^{-1}$) because its volume is 77% of the Lake 303 volume. Food grade phosphoric acid (Univar) was added weekly to barrels on the shoreline and allowed to gradually drain by gravity into the littoral zone. These whole-lake concentrations are comparable to amounts added to experimentally fertilized Lake 227 (16.6 $\mu$g l$^{-1}$ wk$^{-1}$) and eutrophic systems such as Hamilton Harbour in Lake Ontario with annual average whole-lake inputs of 6.4 $\mu$g l$^{-1}$ wk$^{-1}$ between 2008 and 2016 although concentrations would be higher in the epilimnion when inputs are confined to the surface mixed layer during stratification (HHRAP 2018). The lakes were sampled monthly at the deepest stations during the ice-free season from mid-May to mid-October 2017–2019. Depth-integrated surface mixed layer (epilimnion in Lake 304 and the water column in Lake 303) and hypolimnitic samples were filtered using GF/C filters (nominal pore size 1.2 $\mu$m) within 1–2 h of collection, refrigerated, and analyzed within 48 h.

2.3. Analytical methods

Analyses for dissolved P, suspended P, nitrite, nitrate, ammonia, total dissolved N, suspended N and chlorophyll $a$ followed standard protocols (Stainton et al 1977, Schindler et al 2008). Inter-laboratory comparisons and rigorous quality control/assurance protocols were used to ensure quality and consistency of the data. Temperature and dissolved oxygen profiles were measured with an RBR XRX-620 with an Aanderaa oxygen optode 4330F. Algal biomass (wet weight), heterocyst counts and taxonomy from integrated surface mixed layer samples were determined via microscopy (Findlay and Kasián 1987).

Total P and total N are the sum of suspended and dissolved values and their standard deviations are calculated as the square root of the squares of the sum of the dissolved and suspended standard deviations.

3. Results and discussion

Dense phytoplankton blooms in Lake 303 and Lake 304 were visible in aerial photographs taken on 1 September, 12 weeks after the first P addition in mid-June (figure 1). Both blooms were dominated by N$_2$ fixing cyanobacteria Dolichospermum sp. The photo of Lake 304 shows what is probably the remnant of the bloom that had peaked several weeks earlier.

The temperature of the top 2 m in both lakes was between 21 and 24 $^\circ$C during July and August but shallow Lake 303 was isothermal with uniform dissolved oxygen above 2 m (see figure S1, available online at stacks.iop.org/ERL/16/064078/mmedia). Small DO gradients of 0.2–0.6 mg l$^{-1}$ between 2 and 2.5 m were detectable by June 10 and continued throughout August and September in Lake 303 which suggests strong influence from sediment respiration and very little turbulence along the sediment/water boundary. While DO in Lake 303 was higher in October than in July and August, it was much more undersaturated in October (63%–74% vs 80%–89% earlier in the year), indicating that supplies from
Figure 1. Lake 303 and Lake 304 are visibly green in these aerial photos taken on 1 September 2019, 18 d after the bloom peaked in Lake 304 and 8 d before the bloom peaked in Lake 303 (see figure 3). Reproduced with permission from ELA.

photosynthesis and mixing from the surface were less able to meet biochemical oxygen demand in the fall.

In contrast, Lake 304 was thermally stratified by May 13 with a metalimnetic dissolved oxygen maximum at 3 m in June and July (figure 2) coinciding with dominance by flagellated algal species (primarily Dinobryon bavaricum Imhof in June and Peridinium wisconsinense Eddy in July). However, the DO maximum disappeared before the cyanobacteria bloom in August. The hypolimnion was anoxic below 4.8 m on 10 June and thereafter the top of the anoxic zone ascended to 3.3 m by 13 August (figure S1) while the thermocline descended from 2.5 to 3.3 m. The hypolimnion of Lake 304 was anoxic before the bloom began and through the post-bloom phase, remaining so until turnover some time after 9 September. DO in Lake 304 was similarly undersaturated in October ranging from 63 to 74%.

The weekly fertilization rate was sufficient to raise surface mixed layer total P concentrations to eutrophic levels. Mean June–October total P concentrations in 2019 were higher than pre-fertilization concentrations in 2017 and 2018 as were total N and chlorophyll a (table 1). Mean pre-fertilization
total P concentrations were low and typical of other lakes at ELA (11 and 15 \( \mu g \text{ l}^{-1} \) in Lake 303, and 11 and 10 \( \mu g \text{ l}^{-1} \) in Lake 304) increasing to means of 30 and 36 \( \mu g \text{ l}^{-1} \), respectively, in 2019, increases of 2–3-fold. Surface mixed layer total P gradually increased from 10 to 15 \( \mu g \text{ l}^{-1} \) (mostly as particulate P, 7–14 \( \mu g \text{ l}^{-1} \)) in May to a maximum of 45 \( \mu g \text{ l}^{-1} \) in both lakes (mostly as particulate P, 40–42 \( \mu g \text{ l}^{-1} \)) which coincided with peak cyanobacteria biomasses (figure 2). Total and particulate P levels declined after the blooms. The increased phytoplankton biomass during the bloom appears to have temporarily

**Table 1.** Average June–October surface mixed layer total P, total N and chlorophyll \( a \) concentrations (\( \mu g \text{ l}^{-1} \)) and N/P ratios by weight (based on six samples per year), and immediately pre-bloom and peak bloom N/P ratios in Lake 303 and Lake 304, 2017–2019. The lakes were fertilized in 2019. Standard deviations are in brackets.

| Year          | Lake 303 |                |                | Lake 304 |                |                |
|---------------|----------|----------------|----------------|----------|----------------|----------------|
|               | Total P  | Total N        | N/P            | Chlorophyll | Total P  | Total N        | N/P            | Chlorophyll |
| 2017          | 11(1.2)  | 432(48)        | 39(48)         | 3(1)      | 10(1)  | 408(34)        | 42(34)         | 3(0.8)      |
| 2018          | 14(3.4)  | 732(169)       | 54(169)        | 10(6)     | 10(1)  | 398(36)        | 40(36)         | 5(6)        |
| 2019          | 30(9)    | 832(317)       | 28(5)          | 18(18)    | 36(11) | 579(160)       | 17(4)          | 15(10)      |
| 2019 pre-bloom| 22       | 519            | 24             | 8         | 34     | 384            | 11             | 2           |
| 2019 peak bloom| 45       | 1272           | 28             | 51        | 50     | 792            | 16             | 25          |
| 2019 P/N ratio| 12       |                |                |           |        |                |                |             |

Figure 2. Surface mixed layer total P (■), particulate P (●) and dissolved P (○) in Lake 303 and Lake 304 in 2019. Fall overturn ended thermal stratification between 9 September and 7 October. Arrow denotes first P addition.
increased the amount of total P in the water column by sequestering it as particulate P.

Total P dynamics differed between the lakes (figure 2). The rate of total P increase was slower in Lake 303 with a peak concentration recorded four weeks after the peak in Lake 304. Surface mixed layer dissolved P remained low in both lakes after P additions began, ranging from 4 to 10 µg l⁻¹ with the exception of 21 µg l⁻¹ on 8 July in Lake 304. The small increases in surface mixed layer total P and dissolved P from September to mid-October in both lakes followed a period of enhanced mixing that promoted overturn in Lake 304 and may have been due to a combination of a drop in productivity and demand for P by macrophytic, benthic and epilithic communities as temperatures cooled to between 9 and 10 °C and daylength declined, as well as mixing from the hypolimnion of Lake 304 and resuspension of littoral sediments. By late October, similar amounts of added total P had been lost from the water columns to the sediments or discharged downstream in both lakes, 88% in Lake 303 and 85% in Lake 304. These percentages are higher than long-term losses of 61%–84% from the P-limited Dorset study lakes in central Ontario (Dillon and Molot 1996). Hypolimnetic dissolved P in Lake 304 was relatively low, ranging from 3.7 to 9.9 µg l⁻¹ (except after the bloom on 9 September when it was 21.4 µg l⁻¹) in spite of early development of anoxia. This, combined with large losses from the water columns, indicate that internal P loading was likely not significant and that sediments were a P sink, similar to Lake 227 (Orihel et al 2017).

Fertilization increased mean seasonal chlorophyll a 2–6-fold (table 1). Pre-fertilization levels were similar between lakes in 2017 but the average chlorophyll a concentration was elevated in Lake 303 in 2018 along with total P and total N. Shallow polymeric lakes at ELA typically have a larger interannual variability.

Cyanobacteria blooms formed in both lakes but the peaks occurred at different times. In Lake 304, the peak cyanobacteria biomass of 2796 µg l⁻¹ wet weight was recorded on 13 August (figure 3). In Lake 303, the bloom had commenced by 13 August but the peak biomass of 28410 µg l⁻¹ was recorded 4 weeks later on 9 September (figure 3). The monthly
Figure 4. Cyanobacteria biomass in the study lakes (●) and five reference lakes (○). Top panel—Lake 303 (●) and polymictic Lake 114 (○). Bottom panel—Lake 304 (●) and thermally stratified Lakes 224, 239, 373, 442 (○).

sampling frequency may have missed the actual peaks. The blooms were dominated by *Dolichospermum* in both lakes (*D. lemmermannii* Usacev in Lake 304, *D. solitaria* v. plantonica Brunthaler in Lake 303). Chrysophytes dominated phytoplankton in both lakes in pre-fertilization 2017 with cyanobacteria <2% of the surface mixed layer biomass of Lake 304 but reached 11% in Lake 303 in August. Peak surface mixed layer summer phytoplankton biomasses in 2017 were much lower with 1650 µg l\(^{-1}\) in Lake 303 and 734 µg l\(^{-1}\) in Lake 304. Cyanobacteria blooms were not observed in unfertilized lakes at ELA in 2019 (figure 4).

The range of peak cyanobacteria biomasses recorded in Lake 303 and Lake 304 (2800–28400 µg l\(^{-1}\)) was similar to epilimnetic peaks recorded in other eutrophic systems in Ontario: 22000 µg l\(^{-1}\) in Lake 227 in 2014, 830–9300 µg l\(^{-1}\) in Sturgeon Bay in 2012 and 2014 (Verschoor *et al* 2017), and 2900–11300 µg l\(^{-1}\) in Hamilton Harbour, Lake Ontario 2014–2016. Hamilton Harbour has been designated an Area of Concern by the Canada–US International Joint Commission in part because of beneficial use impairment by cyanobacteria blooms (Hiriart-Baer *et al* 2016). The blooms in Lakes 303 and 304 would probably be cause for management concern if they had occurred naturally in recreational lakes or in lakes serving as drinking water supplies.

Mean surface mixed layer total N in June–October was much higher compared to pre-fertilization levels in 2017 and 2018 (table 1), and it doubled in 2019 during the blooms from spring levels. Dissolved inorganic N (DIN) was depleted by the beginning of the blooms with concentrations of 19 and 21 µg l\(^{-1}\) in Lake 303 and Lake 304, respectively, primarily as ammonia. Surface mixed layer total N, dissolved N (organic and inorganic) and DIN concentrations in 2019 declined during the summer, reversing direction when the blooms started (figure 4). DIN depletion at the beginning of the blooms followed by large increases in total N and heterocyst abundance coinciding with increases in cyanobacteria biomass.
strongly infers the importance of N$_2$ fixation to inputs of total N during the bloom (figure 5). No explanation other than N$_2$ fixation can account for the doubling of N mass within two weeks in these lakes. First of all, the N doublings occurred almost four weeks apart, and each doubling was strongly coincident with increases in cyanobacteria biomass and heterocyst abundance. N$_2$ fixation rates and heterocyst abundance are strongly correlated in nearby Lake 227 (Findlay et al 1994, Higgins et al 2018) and the Lake 227 regression accounted for 67% of the N doubling during the bloom in Lake 303. Second, the increases in N mass were not due to an increase in DIN or DON but in PN (figure 5) so internal loading is not a valid explanation. Although the hypolimnion in Lake 304 was anoxic by 10 June, hypolimnetic DIN decreased in the until the bloom began in August after which there was only a very modest increase which did not

**Figure 5.** Surface mixed layer total N (■), particulate N (●) and DIN (○) in 2019 in Lake 303, epilimnion of Lake 304 and hypolimnion of Lake 304. Arrow denotes first P addition.
bring DIN back to early summer levels by the time the bloom peaked (figure 5). The largest increase in DIN in bottom waters occurred after the bloom peaked which was probably caused by mineralization of senescing, settling cells. Third, no large precipitation or major wind events were recorded in the two weeks prior to the bloom peaks that might have transported large amounts of N to the lakes from the watershed or resuspended sediment N. DON is by far, the largest N input from N-starved forest soils and wetlands, especially in the growing season, and DON did not increase. PN contributes only 5% to annual N loads to ELA lakes and is commonly ignored in N lake budgets (Parker et al 2009). Given the lake volumes, direct atmospheric deposition of N to the lake surface during a storm event or N input from the terrestrial catchment could not provide enough N to double the N mass during a storm in the growing season.

Dynamics of surface mixed layer total N to total P ratios by weight (TN/TP) and surface mixed layer particulate N to particulate P ratios by weight (PN/PP) suggest that N$_2$ fixation was a large source of N during the blooms. Large increases in both ratios were strongly associated with large increases in heterocyst abundance and cyanobacteria biomass (figures 3 and 6). TN/TP dropped in the early part of the season as P was added, in Lake 303 from 42 in mid-May to a minimum of 22 in early July, increasing slightly to 28 in September and 36 in early October despite the continuing addition of P (table 1, figure 6). In Lake 304, TN/TP dropped from 60 in mid-May to a minimum of 11 in early July, increasing to 16 in August and September and then 19 in early October. Without these N inputs during the blooms, the N/P ratios would probably have continued to decline. Reversal of the declines in N/P in Lakes 303 and 304 coincided with large increases in heterocyst abundances (figure 6). The ratios continued to increase after the bloom peaked because total P declined faster than total N. PN/PP increased two-fold from values in early June to early July to maximum levels that coincided with peak cyanobacteria biomasses and heterocyst abundances (figures 3 and 6). PN/PP increased from 5.5–5.9 to 10.5 in Lake 304 and 10.1–11.7 to 23.3 in Lake 303.

Mineralization of senescent phytoplankton started during the blooms and continued post-bloom in Lake 304. Total N, dissolved N and DIN in the hypolimnion declined until the bloom started, then increased during the bloom, reaching a peak one
month after the bloom (figures 3 and 5). Total P in the epilimnion and hypolimnion of Lake 304 were similar until after the bloom in September when hypolimnetic total P total increased while epilimnetic total P decreased. Hypolimnetic dissolved P in Lake 304 doubled from 10 µg l⁻¹ on 13 August to 21 µg l⁻¹ on 9 September. A post-bloom mineralization phase also occurred in Lake 303 judging by the increase in dissolved N and DIN in the water column in October as total N declined.

Faster buildup of total P in the water column was associated with earlier bloom onset and it is possible that the rates of total P accumulation were influenced by differences in lake morphology. Well-mixed Lake 303 received 2 kg of P per week for a targeted whole-lake increase of 13.3 µg l⁻¹. The epilimnion of Lake 304 received 1.54 kg per week for a similar whole-lake concentration but the added P was confined to the epilimnion, much less than the whole-lake volume. For example, the concentration of added TP above background in the epilimnion of Lake 304 would have been 36.7 µg l⁻¹ on 10 June and 16.0 µg l⁻¹ on 9 September when the epilimnion depth was 1.3 and 3.8 m, respectively, rather than 13.4 µg l⁻¹ if the lake had been well mixed. Therefore, the more rapid total P buildup and earlier bloom onset in Lake 304 may have been a consequence of differences in the volumes of the receiving layers. Another explanation lies in competition for P between phytoplanktonic and non-phytoplanktonic communities. Lake 303 is shallower with 68% of its sediment area at depths less than 2 m, compared to 30% in Lake 304. Hence, phytoplankton in Lake 303 may have encountered more competition for P from relatively large non-planktonic macrophyte and epibenthic, epilithic and epiphytic algal communities which might have lowered the rate of total P buildup in the water column compared to Lake 304.

4. Implications and conclusions

The whole-lake fertilization of Lakes 303 and 304 clearly demonstrates that large cyanobacteria blooms can rapidly develop under high P loading without anthropogenic N inputs and without recycling of anthropogenic (legacy) N stored in sediments. The lakes turned visibly green 12 and 9 weeks after fertilization began. The P fertilization rates of 13.3–13.4 µg l⁻¹ week⁻¹ were high enough to increase peak TP concentrations 2–3-fold, lower the TN/TP ratios before the blooms began and cause dense, month-long blooms of pelagic N₂-fixing Dolichospermum species which doubled the N content in both lakes during the blooms via N₂ fixation. The assumption that legacy N was unimportant in 2019 is reasonable based on the work of Paterson et al (2011) and Higgins et al (2018) on nearby fertilized Lake 227. They concluded that although Lake 227 was fertilized with N for 20 years, legacy N had little impact two decades years after N fertilization stopped. Lakes 303 and 304 were fertilized with N for 2 and 3 years, respectively, and the current experiment was conducted 35 years after the last N fertilization.

This study replicates one of the major conclusions of the latter part of the Lake 227 study (the 30 year N removal phase), that blooms do not need anthropogenic N fertilizer to develop in P-enriched lakes (Schindler et al 2008, Higgins et al 2018), partially addressing the lack of replication criticism, and demonstrating that bloom onset is relatively rapid without added N. If blooms do not need anthropogenic N to form, then logically, management programs that remove all anthropogenic N from a eutrophic lake will likely not eliminate blooms, although they might lower eukaryotic algal biomass and annual lake productivity if cyanobacteria blooms are relatively brief. It follows that complete elimination of cyanobacteria blooms by nutrient reductions cannot be achieved without P controls. Complete prevention is one end of a mitigation continuum and while partial mitigation via N removal remains a possibility, the evidence is not supportive of major mitigation by N removal. Benchtop experiments with batch cultures of heterocystous cyanobacteria show no major differences in biomass yields with and without inorganic N when trace metal supplies are adequate (Molot 2017, Molot et al 2021) nor did the blooms in Lake 227 become dramatically smaller after N additions stopped (Schindler et al 2008) — in fact, annual peak cyanobacteria biomass increased after high nitrate additions were lowered after 1975 which is consistent with the sediment redox hypothesis of Molot et al (2014, 2021). Of course, there are reasons other than bloom prevention for implementing N controls such as inorganic N toxicity, cyanotoxins and marine eutrophication (Diaz and Rosenberg 2008, Gobler et al 2016, Wagner et al 2021) but managers addressing these issues should not necessarily expect to simultaneously prevent freshwater blooms with aggressive N removal. Bloom prevention in eutrophic lakes can only be achieved through P controls.

Additional replication of whole-lake P-only fertilization experiments in regions with different climates and geology from ELA are desirable to generate confidence in the findings. However, since the bloom-forming cyanobacteria genera in artificially fertilized Lakes 303, 304 and 227, Aphanizomenon and Dolichospermum, are common to many N-limited eutrophic lakes globally, it is clear that fertilized ELA lakes are not so unusual or unique, despite their soft water nature and northern location, that findings elsewhere will be completely contradictory. Indeed, similar genera form very large blooms in the north basin of Lake Winnipeg located 500 km to the northwest (Kling et al 2011) and bloom incidence has been growing in soft water lakes in Ontario (Winter et al 2011).
To be defined as successful, a eutrophication management program should satisfy two distinct objectives: phytoplankton communities should be dominated by non-harmful eukaryotic algae rather than cyanobacteria and phytoplankton biomass should be acceptably low throughout the ice-free season. Although cyanobacteria blooms may not last long, care must be taken to highlight such events and not rely only on ice-free average nutrient and chlorophyll concentrations as proxies of lake behavior since both seasonal averages and shorter-lived cyanobacteria bloom events are important management concerns.

**Data availability statement**

All data that support the findings of this study are included within the article (and any supplementary files) and are available at https://doi.org/10.5683/SP2/Q7FYSI.

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