

# Nitrogen fixation may not balance the nitrogen pool in lakes over timescales relevant to eutrophication management

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## Abstract

We explored multiyear linear trends in nutrient concentrations, nitrogen (N):phosphorus (P) ratio, and phytoplankton biomass within the 37-yr, whole-ecosystem nutrient enrichment experiment in Lake 227 of the Experimental Lakes Area, Canada. Based on experimental conditions, data were divided into subsets, which included (1) the period from 1969 to 1989 when the lake was fertilized with both N and P; (2) the period from 1990 to 2005 when the lake was fertilized with P alone; and (3) the period from 1997 to 2005 when the lake was fertilized with P alone and which also postdated a food web manipulation experiment, which left the lake without fish. After N fertilization was halted in 1990, total N concentrations decreased, which resulted in a decrease in the ratio of total N to total P and suggested increasing N deficiency. Chlorophyll *a* concentration decreased over this same period. Phytoplankton biomass ( $\text{mg m}^{-3}$ ) was highly variable during the food web manipulation experiment but exhibited a clear decrease from 1997 to 2005, which was the longest period of monotonic change in phytoplankton biomass over the entire 37-yr study. Collectively, these results suggest that Lake 227 has become increasingly N-limited since N fertilization was halted and indicate that N fixation by cyanobacteria was not sufficient to offset the decrease in external N inputs to Lake 227. Furthermore, phytoplankton biomass decreased in response to decreased N availability, suggesting that the degree of eutrophication can be controlled by managing N inputs concurrently with P.

The idea that phosphorus (P) ultimately controls primary productivity in lakes has generated support for watershed management programs that do not address nitrogen (N) inputs (Schindler et al. 2008). This recommendation has stimulated a flurry of debate regarding the need to manage both N and P, or just P, to control eutrophication in diverse aquatic systems (Conley et al. 2009; Paerl 2009). The P-only management approach is based on the premise that excess N derived from N fixation will offset imbalanced inputs of N and P from a watershed. But over what timescale does this process occur, and how relevant is this to aquatic ecosystem management?

Cyanobacterial N fixation in temperate lakes is generally restricted to summer months (Howarth et al. 1988; Scott et al. 2008; Marcarelli and Wurtsbaugh 2009). Warm water maximizes microbial metabolism and often supports highest annual biomass, which increases nutrient demand in the pelagic zone and draws down inorganic nutrient pools (Paerl and Huisman 2008). When the N:P of inorganic nutrient pools is low, this drawdown can exhaust inorganic N from the water column, even in eutrophic lakes with very high spring nitrate ( $\text{NO}_3^-$ ) concentrations, and initiate N fixation by cyanobacteria (Scott et al. 2008, 2009).

In freshwater biofilms, cyanobacterial N fixation can alleviate N limitation within days to weeks as fixed N accumulates in the biofilm matrix (Scott et al. 2007). However, this response is not as obvious in the pelagic zone of lakes. In fact, many studies have indicated that N fixation by phytoplankton cannot compensate for N deficiency within a single summer (*see* review by Lewis

and Wurtsbaugh 2008), in large part because this process is controlled by multiple physical–chemical factors in addition to N:P (Paerl 1990, 2009).

Another mechanism by which N fixation could alleviate N limitation is if fixed N accumulated within a lake over several years, ultimately increasing N concentrations to levels that would increase the frequency and severity of P limitation. Schindler et al. (2008) concluded that fixed N accumulated in the sediments of Lake 227 of the Experimental Lakes Area (ELA), Canada, over a multi-annual timescale (Schindler et al. 1987), which led to increased N regeneration rates from epilimnetic sediments in subsequent years, decreased the demand for new N fixation by phytoplankton (Hendzel et al. 1994), and essentially alleviated phytoplankton N limitation.

Although sediments can be an important source of N in many aquatic systems (Fulweiler et al. 2007; McCarthy et al. 2007), many lakes can have a small percentage of sediments in contact with the epilimnion, so that the contribution of N regenerated from sediments influencing primary production would also be relatively small (Fee 1979). Furthermore, epilimnetic sediments can exhibit very high denitrification rates because of warm water temperatures and coupled nitrification–denitrification occurring at an oxygenated sediment–water interface (Saunders and Kalff 2001). Therefore, efficient denitrification in epilimnetic sediments could offset the accumulation of fixed N. Finally, sediments may also release P into the epilimnion (Anderson and Ring 1999), so the ratio of N to P released from sediments also affects the N:P of a lake. Collectively, this information suggests that sediments may or may not effectively accumulate fixed N and that N release into the

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epilimnion may or may not alleviate phytoplankton N limitation in a lake.

Using data obtained from Schindler et al. (2008), we tested for multiyear linear trends in total N, total P, the ratio of total N to total P (TN:TP), and phytoplankton biomass within the 37-yr experimental period in Lake 227. We hypothesized that the elimination of N fertilization in 1990 caused the N pool in the lake to decrease, which subsequently decreased phytoplankton biomass. We predicted that total N concentrations decreased in years after N fertilization was halted without a concomitant decrease in total P, which decreased TN:TP and increased the severity of N limitation. We also predicted that phytoplankton biomass, measured either directly or as chlorophyll *a* (Chl *a*) concentration, decreased after N fertilization was halted. Overall, the purpose of this study was to evaluate if cyanobacterial N fixation could sufficiently balance N and P concentrations in Lake 227 over multi-annual timescales.

## Methods

Data presented in this paper were reported by Schindler and colleagues from Lake 227 in ELA (Schindler et al. 2008). Lake 227 is located in northwestern Ontario, Canada, and has an area of 0.05 km<sup>2</sup> and a mean depth of 4.5 m. The lake was fertilized with both N and P from 1969 to 1989 (110–308 kg N yr<sup>-1</sup> and 19.8–24.8 kg P yr<sup>-1</sup>; Schindler et al. 2008), then fertilized with only P from 1990 to 2005 (23.6–31.9 kg P yr<sup>-1</sup>; Schindler et al. 2008). A food web manipulation experiment was also conducted in Lake 227 from 1993 to 1996 (Elser et al. 2000). Northern pike (*Esox lucius*) were added to the lake in 1993 and 1994, which resulted in the elimination of all the forage fishes (*Pimephales promelas*, *Semotilus margarita*, *Phoxinus eos*, and *Phoxinus neogaeus*) in the lake by 1996. The pike were removed in 1996, and the lake has been without fish since that time.

Annual average total N concentration, total P concentration, TN:TP, Chl *a* concentration, phytoplankton biomass, and the Chl *a*:biomass ratio were gathered from figs. 2–4 in Schindler et al. (2008) by printing graphs at 4× their original size and measuring the distance of each observation from the x-axis to the nearest half millimeter. The data were divided into three subsets: (1) the period when the lake was fertilized with both N and P (1969–1989); (2) the subsequent period when the lake was fertilized with P alone (1990–2005); and (3) the period following the food web manipulation experiment when the lake was fishless and fertilized with P alone (1997–2005). We predicted that total N concentrations, TN:TP, Chl *a* concentration, and phytoplankton biomass in Lake 227 decreased after 1990 when N additions were halted.

To test these predictions, linear regression analyses were conducted (SAS 9.1) between year and each variable for the three data subsets (1969–1989; 1990–2005; 1997–2005) and for all 37 yr of data. In reporting trends from linear regression analyses, we required that the *p* value for slopes be ≤ 0.1 because of the limited number of observations included in the analysis (as few as nine in the post-food

web experiment period). Regression analysis was conducted on the post-food web experiment period when analyses of the P-alone fertilization period were not statistically significant at *p* ≤ 0.1.

## Results

Total P increased in Lake 227 by  $1.3 \pm 0.7 \mu\text{g P L}^{-1} \text{ yr}^{-1}$  from 1969 to 1989 when the lake was fertilized with both N and P (Fig. 1A). There was no trend in total P from 1990 to 2005 ( $F_{1,14} = 0.271$ ,  $p = 0.611$ ), from 1997 to 2005 ( $F_{1,7} = 3.396$ ,  $p = 0.108$ ), or over the entire 37-yr experimental period ( $F_{1,35} = 0.247$ ,  $p = 0.622$ ). Total N concentrations increased by  $25 \pm 7.6 \mu\text{g N L}^{-1} \text{ yr}^{-1}$  from 1969 to 1989, when the lake was fertilized with both N and P, but decreased by  $21 \pm 5.6 \mu\text{g N L}^{-1} \text{ yr}^{-1}$  from 1990 to 2005, when the lake was fertilized with only P (Fig. 1B). The total N accumulation rate from 1969 to 1989 was statistically equivalent to the total N loss rate from 1990 to 2005, leaving no long-term trend in total N over the entire 37-yr experimental period ( $F_{1,35} = 0.655$ ,  $p = 0.424$ ). There was no trend in TN:TP over the entire 37-yr experiment ( $F_{1,35} = 0.040$ ,  $p = 0.844$ ) nor from 1969 to 1989 ( $F_{1,19} = 0.002$ ,  $p = 0.962$ ). However, TN:TP decreased from 1990 to 2005 (Fig. 1C). The observed change in TN:TP over the last 15 yr of the study was the result of stable total P concentrations and declining total N concentrations. From 1996 to 2005, TN:TP was greater than the 37-yr mean in only 2 yr, and in both cases the value was only slightly higher.

Chl *a* concentrations decreased at a rate of  $0.8 \pm 0.3 \mu\text{g L}^{-1} \text{ yr}^{-1}$  from 1990 to 2005 (Fig. 2A). However, there was no trend in Chl *a* concentration from 1969 to 1989 ( $F_{1,19} = 0.118$ ,  $p = 0.735$ ) or over the entire 37-yr experiment ( $F_{1,35} = 1.603$ ,  $p = 0.214$ ). There was no trend in phytoplankton biomass over the entire 37-yr experiment ( $F_{1,35} = 0.050$ ,  $p = 0.824$ ) or in the periods from 1969 to 1989 ( $F_{1,19} = 0.939$ ,  $p = 0.345$ ) and 1990 to 2005 ( $F_{1,14} = 1.192$ ,  $p = 0.293$ ). However, a trend of decreasing phytoplankton biomass was apparent in the years following the food web manipulation experiment (Fig. 2B). The period of decreasing phytoplankton biomass from 1997 to 2005 was the longest period of monotonic change in phytoplankton biomass over the 37-yr experimental period. There was no trend in the Chl *a*:biomass ratio from 1969 to 1989 ( $F_{1,19} = 1.638$ ,  $p = 0.217$ ), from 1990 to 2005 ( $F_{1,14} = 1.008$ ,  $p = 0.333$ ), or from 1997 to 2005 ( $F_{1,7} = 0.083$ ,  $p = 0.782$ ). However, Chl *a*:biomass decreased by  $0.09 \pm 0.04 \text{ yr}^{-1}$  over the entire 37-yr experiment (Fig. 2C).

## Discussion

Phytoplankton N limitation may be common in many eutrophic lakes because of imbalanced inputs of N and P coming from anthropogenic sources (Downing and McCauley 1992). But, can N fixation offset N limitation and maintain high phytoplankton biomass? Schindler et al. (2008) concluded that, over a period of several years, N fixation effectively alleviated N limitation in Lake 227, and that P fertilization alone “kept the lake eutrophic, with no

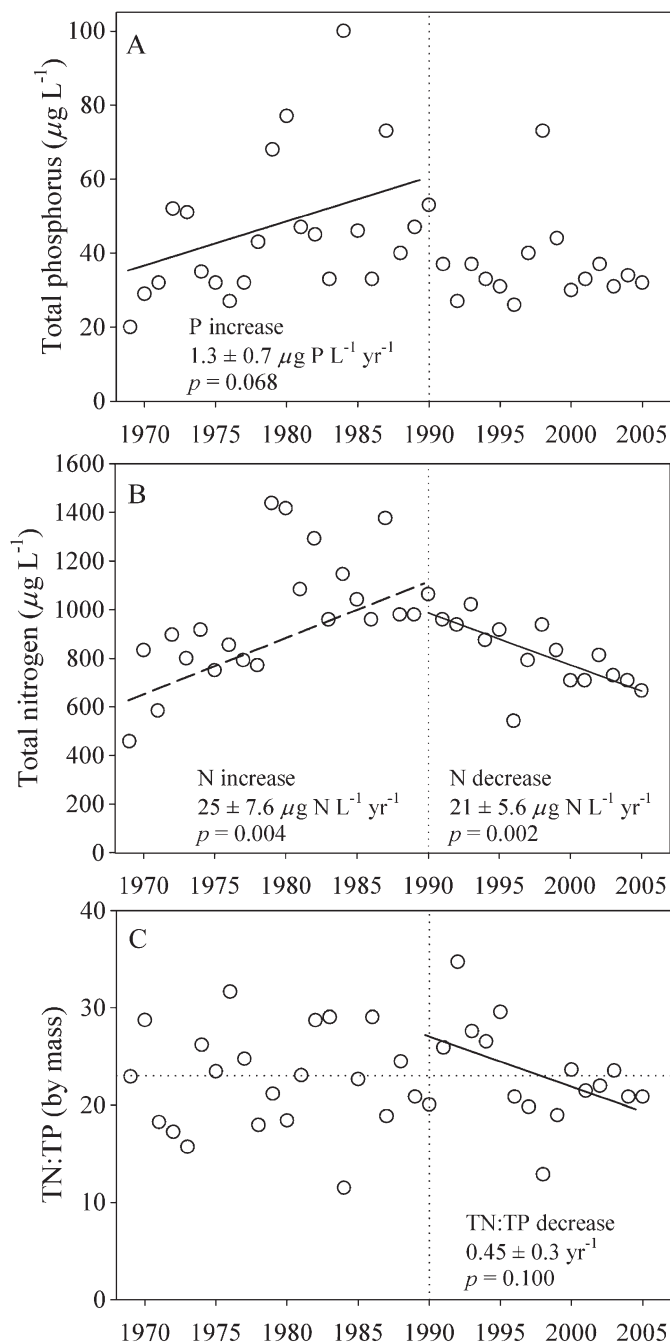


Fig. 1. Reexamination of the long-term (A) total P, (B) total N, and (C) TN:TP ratio data presented by Schindler et al. (2008). Vertical dotted line in all panels represents the year in which lake fertilization with N was halted. Horizontal dotted line in (C) represents the 37-yr mean TN:TP.

substantial changes in biomass of phytoplankton.” Our reexamination of Lake 227 data does not support this conclusion. Phytoplankton biomass has declined since 1997 (Fig. 2B), and Chl *a* concentration has declined since 1990 (Fig. 2A) as TN:TP decreased (Fig. 1C) and Lake 227 became increasingly N-limited.

The apparent absence of a trend in total N over the entire 37-yr dataset is driven by equivalent rates of total N

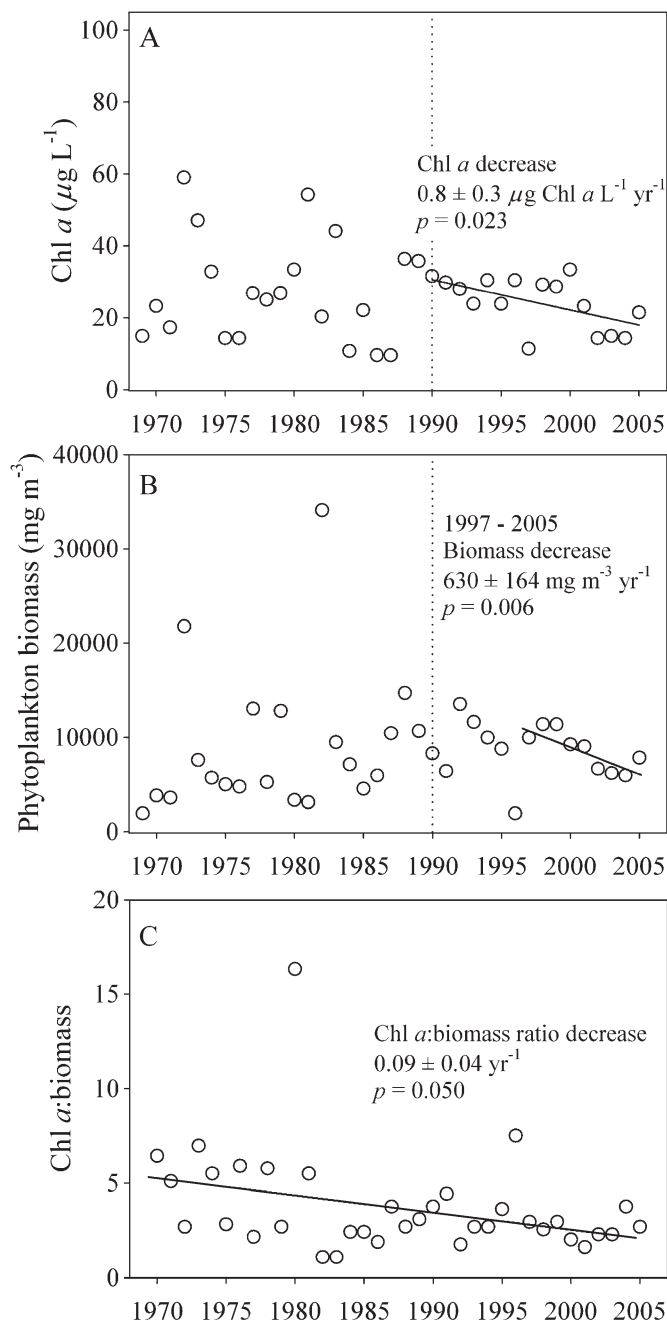


Fig. 2. Reexamination of the long-term (A) Chl *a*, (B) phytoplankton biomass, and (C) chlorophyll:biomass ratio data presented by Schindler et al. (2008). Vertical dotted line (A, B) represents the year in which lake fertilization with N was halted.

increase from 1969 to 1989 and total N decrease from 1990 to 2005. This suggests that, when the lake was fertilized with P alone (1990–2005), sediment N burial, washout, and/or denitrification decreased the N pool in the lake at a rate equivalent to its accumulation when N and P were added in concert (1969–1989). When normalized to epilimnetic volume and corrected for annual fixed N inputs, the N loss rate in Lake 227 from 1990 to 2005 was  $\sim 1 \text{ g N m}^{-2} \text{ yr}^{-1}$ . Denitrification in Lake 227 has been estimated to be as high as  $5\text{--}7 \text{ g N m}^{-2} \text{ yr}^{-1}$  (Chan and

Campbell 1980). Although more work is needed to refine the denitrification estimates, these rates indicate that the observed decrease in Lake 227 total N may be largely the result of permanent N removal.

Levine and Schindler (1992) suggested that denitrification exhibited less influence on epilimnetic N:P than sediment N regeneration in Lakes 303 and 302S of ELA, particularly when external N:P supply was also low. They argued that denitrification was limited by availability of  $\text{NO}_3^-$ , which was rapidly consumed by phytoplankton when the N:P supply favored N limitation. However, that study was conducted over a very short time period compared to the multi-annual dataset from Lake 227. The decrease observed in TN, TN:TP, Chl *a*, and phytoplankton biomass in Lake 227 after halting N fertilization suggests that the frequency and magnitude of phytoplankton N limitation is probably increasing as N is slowly depleted from the lake. This probability is supported by a recent surge in N fixation rates, which were not accompanied by an increase in cyanobacterial biomass (Schindler et al. 2008). Nitrogen inputs from cyanobacterial N fixation have not been sufficient to alleviate the imbalance in TN:TP caused by both external P additions and internal N loss through denitrification and other permanent N removal mechanisms, as evidenced by the decreasing TN:TP from 1990 to 2005 (Fig. 1C).

It is worth noting that the ratio of total N accumulation to total P accumulation from 1969 to 1989 was  $19.3 \pm 8.3$  (by mass). Thus, N and P were accumulating at rates that would indicate P limitation of algal growth, even though N and P inputs were imbalanced toward N limitation (N:P  $\approx$  5) from 1975 to 1989 (Schindler et al. 2008). This suggests that N fixation may have kept up with excess P inputs from 1975 to 1989, when the imbalance between N and P inputs was less severe. However, N fixation in Lake 227 from 1990 to 2005 failed to counteract N deficiency after N fertilization was halted in 1990.

The capacity of N fixation to alleviate N limitation in lakes is highly variable. In Lake 261 of ELA, which is located only 3 km from Lake 227, N fixation was not detectable during the 3 yr of P fertilization, even though the N:P supply ratio was imbalanced toward N limitation (Flett et al. 1980). Furthermore, only modest increases were observed in phytoplankton biomass and primary production in Lake 261 when it was fertilized with P alone (Fee 1979; Lewis and Wurtsbaugh 2008). In another example, total P concentrations in Lake Okeechobee, Florida, have steadily increased over the last 30 yr, and the lake has experienced frequent cyanobacterial blooms as TN:TP decreased (Havens et al. 2003). However, annual average total N concentration in Lake Okeechobee has remained unchanged because of sediment resuspension, which decreases euphotic depth and limits the effectiveness of cyanobacterial N fixation as a N source (Havens et al. 2003). But N fixation in other lakes can counteract N deficiency, even over an annual cycle. For example, imbalanced watershed N:P inputs ( $\sim$  11 by moles) into Lake Waco, Texas, were balanced by inputs from planktonic N fixation (whole-lake N:P inputs  $\approx$  20 by moles) over each summer in 2003 and 2004 (Scott et al.

2008). Thus, N fixation is capable of adding large quantities of fixed N to balance N:P within any given year when conditions are favorable.

The constraints on N fixation can differ drastically among different aquatic systems (Howarth et al. 1988; Paerl 1990). Secondary limitation by micronutrients (Wurtsbaugh and Horne 1983) or light (Lewis and Levine 1984), and/or physiologic limitations on the number of heterocysts per cyanobacterial filament (Lewis and Levine 1984), may limit N fixation by cyanobacteria. The food web manipulation experiment in Lake 227 clearly demonstrated that reductions in phytoplankton biomass caused by increased zooplankton grazing, and changes in the relative availability of N and P controlled by consumer-driven nutrient recycling, can also control cyanobacterial N fixation rates (Elser et al. 2000). Another important consideration for ecosystem-scale N:P balance is that there is no obvious mechanism by which fixed N inputs would “overshoot” balanced conditions with P (Sterner 2008). In other words, cyanobacterial N fixers will not continue to fix N once the N pool has been sufficiently balanced. Therefore, removal mechanisms, such as denitrification, can offset inputs in any given year, leading to persistent N-limiting conditions. Previous work on benthic N fixation supports this idea (Scott et al. 2007). Recently fixed N accumulated in wetland metaphyton, rapidly causing a shift to stronger P limitation during a growing season. Nitrogen fixation rates quickly declined as fixed N accumulated in biofilms (Scott et al. 2007). Because metaphyton grows at the water surface, light availability for this community was very high. Also, biofilm matrices provide an ideal zone for nutrient retention and recycling (Wetzel 1993), which leads to effective reutilization of fixed N (Scott et al. 2007) and perhaps even micronutrients. Comparable mechanisms are not apparent for planktonic systems when fixed N is transported to sediments, where it may then be buried or denitrified.

Schindler et al. (2008) also conclude that “controlling N inputs could actually aggravate the dominance of N-fixing cyanobacteria,” suggesting that an increase in the occurrence of cyanobacterially derived water quality problems (Huisman et al. 2005) may be linked to decreased external N inputs. However, failure to control external N inputs also may exacerbate the proliferation of non-N-fixing cyanobacteria, such as *Microcystis*. This genus is highly competitive for reduced, inorganic N forms (Blomqvist et al. 1994), forms toxic surface blooms affecting public health and drinking water supplies (Ye et al. 2009), and accumulates P during sedimentary phases (Ahn et al. 2002). The ability to accumulate and store P leads to high N assimilation without comparable P acquisition during bloom phases, resulting in decreased TN:TP (Ahn et al. 2002). In Lake Erie, reductions in P loading provided temporary mitigation of mostly N-fixing cyanobacteria blooms, but annual blooms of *Microcystis* have occurred since 1995, despite no increases in external P loading (Rinta-Kanto et al. 2009). Further, dominance of a cyanobacterial community by species capable of N fixation does not ensure active N fixation. This was the case in a lake in Vermont, where the dominant cyanobacteria were

N fixers, but N acquired via N fixation was minimal (< 9%) compared to ammonium uptake (> 80%; Ferber et al. 2004). Thus, cyanobacteria capable of N fixation may still dominate in some cases because they also prefer ammonium as their inorganic N source, but can rapidly adjust to diurnal or short-term episodes of N scarcity by fixing N.

Although our analysis demonstrated that a decrease in external N inputs to Lake 227 increased N deficiency and decreased phytoplankton biomass, Lake 227 has remained eutrophic. Therefore, the true effects of discontinuing N fertilization will not be known until the N pool and phytoplankton biomass reach a new equilibrium. It is interesting to note that the amount of Chl *a* per unit phytoplankton biomass has steadily decreased over the 37-yr experiment (Fig. 2C). Schindler et al. (2008) pointed out that Chl *a*:biomass is higher in Lake 227 when N is in excess of algal requirements, which seems reasonable given the importance of N in chlorophyll molecular structure. Furthermore, this pattern may help explain the time lag seen between the initiation of Chl *a* decline after 1990 and the initiation of phytoplankton biomass decline after 1997. Magnification of N deficiency caused by the continued downward trend in total N and continued P fertilization helps explain the continued decrease of Chl *a*:biomass.

What does this mean for nutrient management at the watershed scale? Our study suggests that eutrophication can be controlled to some extent by reducing N inputs and supports the idea that combined N and P management is needed for controlling eutrophication in lakes, rivers, and estuaries along the freshwater to marine continuum (Lewis and Wurtsbaugh 2008; Conley et al. 2009; Paerl 2009). We recognize that reducing P inputs has the greatest potential for reducing phytoplankton biomass in most lakes. However, N inputs also stimulate algal biomass and primary production, particularly when combined with elevated P inputs (Fee 1979; Elser et al. 2007). Therefore, both N and P should be considered in watershed nutrient management plans.

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#### References

- AHN, C.-Y., A.-S. CHUNG, AND H.-M. OH. 2002. Rainfall, phycocyanin, and N:P ratios related to cyanobacterial blooms in a Korean large reservoir. *Hydrobiologia* **474**: 117–124, doi:10.1023/A:1016573225220
- ANDERSON, F. O., AND P. RING. 1999. Comparison of phosphorus release from littoral and profundal sediments in a shallow, eutrophic lake. *Hydrobiologia* **408**: 175–183, doi:10.1023/A:1017027818233
- BLOMQUIST, P., A. PETTERSSON, AND P. HYENSTRAND. 1994. Ammonium-nitrogen: A key regulatory factor causing dominance of non-nitrogen-fixing cyanobacteria in aquatic systems. *Arch. Hydrobiol.* **132**: 141–164.
- CHAN, Y. K., AND N. E. R. CAMPBELL. 1980. Denitrification in Lake 227 during summer stratification. *Can. J. Fish. Aquat. Sci.* **37**: 506–512, doi:10.1139/f80-065
- CONLEY, D. J., AND OTHERS. 2009. Controlling eutrophication: Nitrogen and phosphorus. *Science* **323**: 1014–1015, doi:10.1126/science.1167755
- DOWNING, J. A., AND E. MCCAULEY. 1992. The nitrogen:phosphorus relationship in lakes. *Limnol. Oceanogr.* **37**: 936–945.
- ELSER, J. J., AND OTHERS. 2000. Pelagic C:N:P stoichiometry in a eutrophied lake: Responses to a whole-lake food-web manipulation. *Ecosystems* **3**: 293–307, doi:10.1007/s100210000027
- , AND OTHERS. 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine, and terrestrial ecosystems. *Ecol. Lett.* **10**: 1135–1142, doi:10.1111/j.1461-0248.2007.01113.x
- FEE, E. J. 1979. A relation between lake morphometry and primary productivity and its use in interpreting whole-lake eutrophication experiments. *Limnol. Oceanogr.* **24**: 401–416.
- FERBER, L. R., S. N. LEVINE, A. LINI, AND G. P. LIVINGSTON. 2004. Do cyanobacteria dominate in eutrophic lakes because they fix atmospheric nitrogen? *Freshw. Biol.* **49**: 690–708, doi:10.1111/j.1365-2427.2004.01218.x
- FLETT, R. J., D. W. SCHINDLER, R. D. HAMILTON, AND N. E. R. CAMPBELL. 1980. Nitrogen fixation in Precambrian Shield lakes. *Can. J. Fish. Aquat. Sci.* **37**: 494–505, doi:10.1139/f80-064
- FULWEILER, R. W., S. W. NIXON, B. A. BUCKLEY, AND S. L. GRANGER. 2007. Reversal of net dinitrogen gas flux in coastal marine sediments. *Nature* **448**: 180–182, doi:10.1038/nature05963
- HAVENS, K. E., R. T. JAMES, T. E. EAST, AND V. H. SMITH. 2003. N:P ratios, light limitation, and cyanobacterial dominance in a subtropical lake impacted by non-point source nutrient pollution. *Environ. Pollut.* **122**: 379–390, doi:10.1016/S0269-7491(02)00304-4
- HENDZEL, L. L., R. E. HECKY, AND D. L. FINDLAY. 1994. Recent changes of N<sub>2</sub>-fixation in Lake 227 in response to reduction of the N:P loading ratio. *Can. J. Fish. Aquat. Sci.* **51**: 2247–2253, doi:10.1139/f94-228
- HOWARTH, R. W., R. MARINO, AND J. J. COLE. 1988. Nitrogen fixation in freshwater, estuarine, and marine ecosystems. 2. Biogeochemical controls. *Limnol. Oceanogr.* **33**: 688–701.
- HUISMAN, J., H. C. P. MATTHIJS, AND P. M. VISSER. 2005. Harmful cyanobacteria. Springer.
- LEVINE, S. N., AND D. W. SCHINDLER. 1992. Modification of the N:P ratio in lakes by in situ processes. *Limnol. Oceanogr.* **37**: 917–935.
- LEWIS, W. M., AND S. N. LEVINE. 1984. The light response of nitrogen fixation in Lake Valencia, Venezuela. *Limnol. Oceanogr.* **29**: 894–900.
- , AND W. A. WURTSBAUGH. 2008. Control of lacustrine phytoplankton by nutrients: Erosion of the phosphorus paradigm. *Int. Rev. Hydrobiol.* **93**: 446–465, doi:10.1002/iroh.200811065
- MARCARELLI, A. M., AND W. A. WURTSBAUGH. 2009. Nitrogen fixation varies spatially and seasonally in linked stream-lake ecosystems. *Biogeochemistry* **94**: 95–110, doi:10.1007/s10533-009-9311-2
- MCCARTHY, M. J., P. J. LAVRENTYEV, L. YANG, L. ZHANG, Y. CHEN, B. QIN, AND W. S. GARDNER. 2007. Nitrogen dynamics and microbial food web structure during a summer cyanobacterial bloom in a subtropical, shallow, well-mixed eutrophic lake (Lake Taihu, China). *Hydrobiologia* **581**: 195–207, doi:10.1007/s10750-006-0496-2
- PAERL, H. W. 1990. Physiological ecology and regulation of N<sub>2</sub> fixation in natural waters. *Adv. Microb. Ecol.* **11**: 305–344.
- . 2009. Controlling eutrophication along the freshwater-marine continuum: Dual nutrient (N and P) reductions are essential. *Estuar. Coasts* **32**: 593–601, doi:10.1007/s12237-009-9158-8

- , AND J. HUISMAN. 2008. Blooms like it hot. *Science* **320**: 57–58, doi:10.1126/science.1155398
- RINTA-KANTO, J. M., AND OTHERS. 2009. The diversity and distribution of toxigenic *Microcystis* spp. in present day and archived pelagic and sediment samples from Lake Erie. *Harmful Algae* **8**: 385–394, doi:10.1016/j.hal.2008.08.026
- SAUNDERS, D. L., AND J. KALFF. 2001. Denitrification rates in the sediments of Lake Memphremagog, Canada-USA. *Water Res.* **35**: 1897–1904, doi:10.1016/S0043-1354(00)00479-6
- SCHINDLER, D. W., R. H. HESSLEIN, AND M. A. TURNER. 1987. Exchange of nutrients between sediments and water after 15 years of experimental eutrophication. *Can. J. Fish. Aquat. Sci.* **44**: 26–33, doi:10.1139/f87-277
- , AND OTHERS. 2008. Eutrophication of lakes cannot be controlled by reducing nitrogen inputs: Results of a 37-year whole-ecosystem experiment. *Proc. Natl. Acad. Sci. USA* **105**: 11254–11258, doi:10.1073/pnas.0805108105
- SCOTT, J. T., R. D. DOYLE, J. A. BACK, AND S. I. DWORKIN. 2007. The role of N<sub>2</sub> fixation in alleviating N limitation in wetland metaphyton: Enzymatic, isotopic, and elemental evidence. *Biogeochemistry* **84**: 207–218, doi:10.1007/s10533-007-9119-x
- , ———, S. J. PROCHNOW, AND J. D. WHITE. 2008. Are watershed and lacustrine controls on planktonic N<sub>2</sub> fixation hierarchically structured? *Ecol. App.* **18**: 805–819, doi:10.1890/07-0105.1
- , J. K. STANLEY, R. D. DOYLE, M. G. FORBES, AND B. W. BROOKS. 2009. River-reservoir transition zones are nitrogen fixation hot-spots regardless of ecosystem trophic state. *Hydrobiologia* **625**: 61–68, doi:10.1007/s10750-008-9696-2
- STERNER, R. W. 2008. On the phosphorus limitation paradigm for lakes. *Int. Rev. Hydrobiol.* **93**: 433–445, doi:10.1002/iroh.200811068
- WETZEL, R. G. 1993. Microcommunities and microgradients: Linking nutrient regeneration, microbial mutualism, and high sustained aquatic primary production. *Neth. J. Aquat. Ecol.* **27**: 3–9, doi:10.1007/BF02336924
- WURTSBAUGH, W. A., AND A. HORNE. 1983. Iron in eutrophic Clear Lake, California: Its importance for algal nitrogen fixation and growth. *Can. J. Fish. Aquat. Sci.* **40**: 1419–1429, doi:10.1139/f83-164
- YE, W., X. LIU, J. TAN, D. LI, AND H. YANG. 2009. Diversity and dynamics of microcystin-producing cyanobacteria in China's third largest lake, Lake Taihu. *Harmful Algae* **8**: 637–644, doi:10.1016/j.hal.2008.10.010

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