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Experimental iron amendment suppresses toxic cyanobacteria in a hypereutrophic lake

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Abstract. The effects of reducing nutrient inputs to lakes and reservoirs are often delayed by hysteresis resulting from internal phosphorus (P) loading from sediments. Consequently, controlling harmful algal blooms (HABs) in many eutrophic ecosystems requires additional management to improve water quality. We manipulated iron (Fe) concentrations in a hypereutrophic lake to determine if Fe amendment would suppress HABs by inhibiting P release from sediments. Our experiment consisted of 15 in situ mesocosms, 12 of which each received a different dose of Fe (ranging from 2 to 225 g/m²); the remaining three were unmanipulated to serve as controls. Iron amendment decreased P accumulation in porewaters and the flux of P from sediments, which significantly lowered P concentrations in the water column. Iron exerted significant dose-dependent negative effects on the biomass of phytoplankton and periphyton, and reduced the dominance of cyanobacteria. Even at the lowest doses, Fe appeared to reduce the toxicity of cyanobacterial blooms, as measured by concentrations of hepatotoxic microcystins. Overall, our findings highlight the potential for Fe treatment as an effective strategy for minimizing HABs in eutrophic lakes and reservoirs. More broadly, our study reinforces the importance of Fe in regulating the trophic state of freshwaters, and the sensitivity of certain ecosystems to changes in Fe supply. Finally, we hypothesize that decreases in natural Fe supplies to lakes associated with anthropogenic activities may worsen outbreaks of toxic cyanobacteria.

Key words: cyanobacteria; eutrophication; freshwater; harmful algal blooms; internal phosphorus loading; iron treatment; lake remediation; microcystin; sediments.

INTRODUCTION

Record-breaking outbreaks of harmful algal blooms (HABs) have been documented in lakes around the world (Carmichael 2008, Hudnell 2010, Taranu et al. 2015). Numerous case histories of lake restoration have demonstrated that reducing phosphorus (P) loading to freshwaters improves water quality (Cooke et al. 2005, Schindler 2012, Dove and Chapra 2015). In many cases, however, recovery is delayed by hysteresis as recycling of P from sediments contributes to the maintenance of HABs (Søndergaard et al. 2013). Here, we describe an experiment designed to reduce the return of P from enriched sediments, potentially hastening the recovery of lakes where external sources of P have been reduced. More generally, our study examines the influence of one of the earth's most common elements, the ubiquitous transition metal iron (Fe), on the trophic state of lakes. Iron plays a complex (and, as we explain here, potentially contradictory) role in regulating the primary productivity of aquatic ecosystems.

Iron may reduce algal growth by precipitating and sequestering P in sediments. In many eutrophic lakes, particularly those with low Fe availability, internal P loading from sediments is a common phenomenon that fuels HABs (Smith and Schindler 2009, Orihel et al. 2015). Although several biogeochemical mechanisms control P cycling across the sediment–water interface (Boström et al. 1988, Smolders et al. 2006), the long-term sequestration of P in lake sediments likely results from the authigenic formation of ferrous [Fe(II)] phosphate minerals (Gächter and Müller 2003, Katsev et al. 2006). The most stable Fe(II) phosphate mineral is vivianite [Fe₃(PO₄)₂·8H₂O], which precipitates in anoxic sediments supersaturated with Fe and P, but is only stable in the absence of sulfide (H₂S) (Nriagu 1972, Murphy et al. 2001). Vivianite has been identified in sediments of freshwater lakes worldwide (e.g., Nembrini et al. 1983, Manning et al. 1991, Fagel et al. 2005).

Conversely, Fe is also an essential micronutrient for nearly all microorganisms, including cyanobacteria (Cornelis and Andrews 2010). Experimental Fe additions can trigger blooms of equatorial marine phytoplankton (Martin et al. 1994, Coale et al. 1996), but there is no evidence that Fe controls primary productivity at the ecosystem-level in freshwater. Instead, bottle-scale experiments have shown that Fe can be a proximate limiting nutrient of algal growth (Wurtsbaugh and Horne

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TABLE 1. Iron treatments of lakes and reservoirs worldwide.

Lake	Country	Area (km ²)	Year	Dosing regime	Iron dose (g Fe·m ⁻² ·yr ⁻¹)	Compound	References
Dordrecht Reservoir	Netherlands	–	1962	Single	–	FeCl ₃	Peelen (1969)
Lake Lillesjon	Sweden	0.04	1975	Single	146	FeCl ₃	Ripl (1976, 1978)
White Lough	Ireland	0.07	1980	Single	11	Fe ₂ (SO ₄) ₃	Foy (1985), Foy and Fitzsimons (1987)
Foxcote Reservoir	England	0.19	1981	Single	10	Fe ₂ (SO ₄) ₃	Hayes et al. (1984), Young et al. (1988), Daldorph (1999)
Lake Vadnais	USA	1.59	1988–1995	Continuous	1–9	FeCl ₃	Walker Jr. et al. (1989), Engstrom (2005)
Lake Groot Vogelenzang	Netherlands	0.18	1989	Single	100	FeCl ₃	Quaak et al. (1993), Boers et al. (1994)
Black Lake	Canada	0.04	1990–1992	Multiple	4–7	FeCl ₃ , Fe ₂ (SO ₄) ₃	Hall et al. (1994)
Lake Krupunder	Germany	0.07	1991	Single	~3	FeClSO ₄	Jaeger (1994)
Lake Groß-Glienicke	Germany	0.68	1992–1993	Single	500	Fe(OH) ₃ , FeCl ₃	Wolter (2010), Kleeberg et al. (2012, 2013), Rothe et al. (2014)
Alte Donau	Austria	1.58	1995	Single	295	FeCl ₃	Donabaum et al. (1999), Dokulil et al. (2011)
Bautzen Reservoir	Germany	5.30	1996–1997	Continuous	19–21	FeCl ₂	Deppe et al. (1999), Deppe and Benndorf (2002)
Maltanski Reservoir	Poland	0.62	2005–present	Multiple	~0.3	Fe ₂ (SO ₄) ₃	Goldyn et al. (2014)
Lake Uzarzewskie	Poland	0.11	2006–2007	Multiple	~0.5	Fe ₂ (SO ₄) ₃	Kowalczewska-Madura et al. (2008), Kozak and Goldyn (2014)
Lac Heney	Canada	12.32	2007	Single	18	FeCl ₃	Carignan (2014)
Lake Durowskie	Poland	1.44	2009–present	Multiple	~0.4	Fe ₂ (SO ₄) ₃	Goldyn et al. (2013, 2014)
Lake Goreckie	Poland	1.05	2010	Multiple	–	Fe ₂ (SO ₄) ₃	Sobczyński et al. (2012)

Note: Year shows year(s) of iron application, iron doses were calculated based on information in studies cited or from authors via personal communication.

1983, Twiss et al. 2000, Downs et al. 2008). In vitro and in vivo experiments have also shown that changes in Fe availability affect the outcome of competition among phytoplankton species (Pollinger et al. 1995, Vuorio et al. 2005, Molot et al. 2010). In particular, cyanobacteria are responsive to Fe given their evolutionary origins in an Fe-rich anoxic ocean (Kranzler et al. 2013) and diazotrophic cyanobacteria have a specific need for Fe in enzymes required for atmospheric N₂ fixation (Sohm et al. 2011).

These contrasting roles of Fe as a sedimentary sink for P and a micronutrient for algae have resulted in two strikingly different paradigms for managing freshwater eutrophication. For over four decades, Fe salts have been used worldwide as a remediation strategy to reduce P concentrations in lakes and reservoirs (Table 1). While this technique has been applied most commonly in Europe, Fe treatment has also been successfully used for remediation of eutrophication in North American lakes, such as Lake Vadnais, Minnesota, USA (Walker Jr. et al. 1989, Engstrom 2005) and Lac Heney, Québec, Canada (Carignan 2014).

Yet, based on the hypothesis that high Fe availability favors cyanobacterial dominance, some authors have recently suggested that Fe loading to lakes should be reduced as a management strategy to control cyanobacterial blooms (Molot et al. 2010, 2014, Xu et al. 2012).

To reconcile these conflicting management paradigms, we conducted an experimental manipulation of Fe loading to a hypereutrophic, polymictic lake (Fig. 1). Like many shallow, nutrient-rich lakes, seasonal releases of P from sediments in our study lake routinely cause intense blooms of toxic cyanobacteria (Orihel et al. 2015). We amended in-lake mesocosms with different doses of Fe and monitored changes in sediment chemistry, nutrient cycling, algal biomass and community composition, and concentrations of the cyanotoxin microcystin. Our a priori hypotheses of how increased Fe supply affects a hypereutrophic lake were: (1) Fe lowers P concentrations in sediment porewaters by reducing the accumulation of H₂S, allowing for the formation of stable Fe(II) phosphate minerals; (2) sequestration of P in Fe(II) minerals reduces internal P

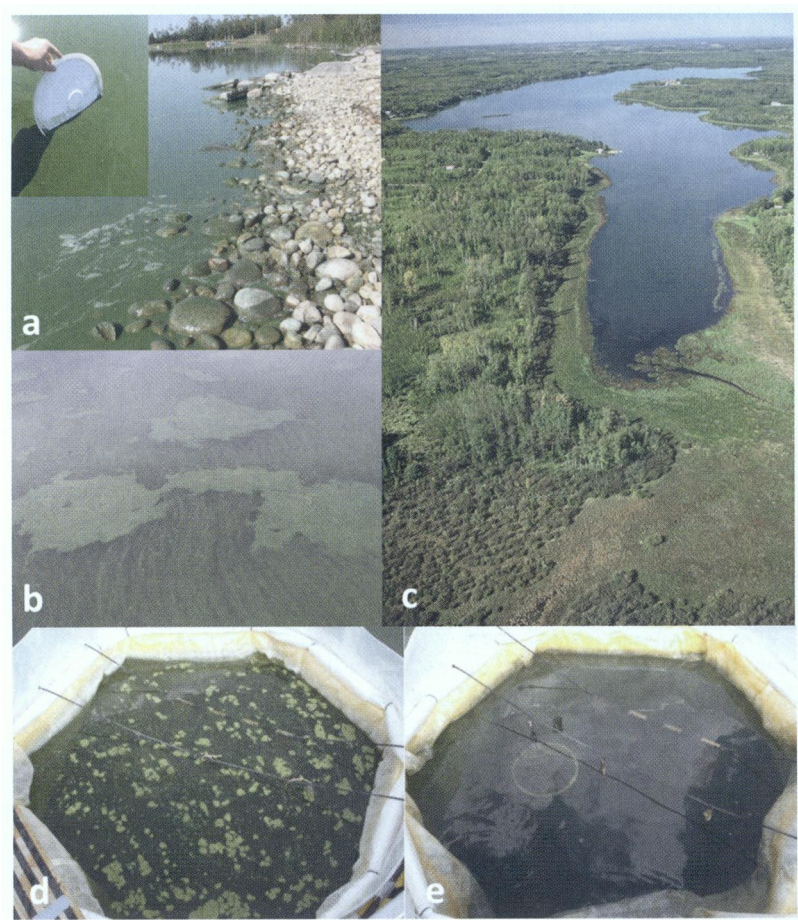


FIG. 1. Like many nutrient-rich ecosystems, Nakamun Lake (Alberta, Canada) experiences intense cyanobacterial blooms that (a) foul its shoreline with green noxious scums and (b) form unsightly floating mats on the lake surface. (c) Aerial view of Nakamun Lake (photo credit: Cows and Fish). (d) Control mesocosm and (e) iron-treated mesocosm in late summer. Photo credit for panels a, b, d, e: Diane Orihel

loading from sediments, which prevents episodic increases in P concentrations, and concomitant declines of nitrogen (N):P ratios, in surface waters (Orihel et al. 2015); (3) less P availability in the water column decreases algal production, while a higher N:P ratio removes the competitive advantage of N_2 fixation by diazotrophic cyanobacteria (Schindler 1977, Schindler et al. 2008); and (4) Fe reduces microcystin concentrations of lakewater by suppressing cyanobacterial blooms. As a potential caveat, if cyanobacteria are Fe-limited, then Fe amendment may stimulate their growth.

METHODS

Study site

Nakamun Lake (53°52′58.42″ N, 114°12′14.16″ W) is a hardwater lake in the Boreal Plains Ecozone of central Canada (Fig. 1c). A thorough description of this lake can be found in Orihel et al. (2015). Briefly, the lake is situated in the Athabasca River drainage basin and the catchment consists mainly of gray luvisol soils. The

surface area of the lake is 3.5 km² and its mean and maximum depths are 4.5 and 8.0 m, respectively. The lake is naturally productive, but residential and agricultural development have contributed to the lake’s current hypereutrophic state (Ballard 2011). Sediments in this lake become anoxic within a few millimeters of the sediment–water interface, are devoid of Fe-P minerals, and release high rates of P under reducing conditions (Orihel et al. 2015).

Mesocosms

Fifteen large enclosure-type mesocosms were installed in Nakamun Lake in May 2009. Each mesocosm (Currie Industries, Winnipeg, Manitoba, Canada) consisted of a 2 m diameter, vinyl-covered Styrofoam (Dow, Midland, Michigan, USA) collar reinforced with a hexagonal aluminum frame, and a 6-m deep, Novathene (Sangir, Vapi, India) wall externally supported by rings of plastic pipes. Mesocosms were anchored in a bay on the north shore over a water depth of 4.5 m. The open bottoms of the mesocosms were embedded into the sediments (~0.5 m

deep) with overlapping rings of sandbags. Small fish inadvertently trapped during installation were removed by pulling a circular net up through the mesocosms.

Based on biweekly measurements taken with a multi-parameter water quality sonde (Hydrolab Series 5 Datasonde Multiprobe; Hach Company, London, Ontario, Canada) from June to October: temperature ranged from 2°C to 23°C, pH ranged from 7.1 to 9.3, and specific conductivity ranged from 370 to 623 $\mu\text{S}/\text{cm}$ in surface waters, and oxidation-reduction potential was usually above +200 mV in bottom waters. The mesocosms experienced multiple thermal stratification events throughout the summer, with a prolonged event beginning in late July (Appendix S1: Fig. S1). During this stratification event, near-hypoxic conditions developed in some mesocosms, but otherwise, bottom waters remained oxic during the open-water season (range: 5.6–14.4 mg/L). Hypoxic conditions also developed in some mesocosms in winter.

Water in the mesocosms was successfully isolated from the lake for the duration of the experiment, as assessed by changes in chloride concentrations. However, two mesocosms (42 and 148 g Fe/m²) had a small degree of mixing with the surrounding lake in September, due to small holes chewed through their walls by muskrats. This may have introduced additional variability, but overall did not impact our conclusions.

Iron additions

Twelve mesocosms were each treated with a different dose of Fe (2, 3, 5, 8, 12, 18, 28, 42, 64, 97, 148, or 225 g Fe/m² sediment). The remaining three mesocosms were not amended with Fe to serve as controls. Treatments were assigned using a spatially stratified random scheme and doses were determined based on pilot studies (Orihel et al. 2015). Standard liquid grade ferric chloride (13.8% \pm 0.7% Fe; Kemira Water Solutions, Varennes, Quebec, Canada) was added over 3 d (6–9 June 2009). Each day, one-third of the total dose was evenly delivered over 10 min by injecting Fe into a stream of lake water pumped into surface waters of each mesocosm. Control mesocosms received the same treatment, but without any Fe amendment. Samples of sediment, water, phytoplankton, and periphyton were collected from all mesocosms over the next 10 months, with additional specialized sampling performed in five “intensive” mesocosms (0, 2, 12, 42, and 225 g Fe/m²).

Sediment sampling

Intact sediment cores were retrieved from all mesocosms using a Glew gravity-driven corer in March 2010. Cores were extruded at 2.5-cm intervals to a depth of 20 cm, and samples were centrifuged ($\sim 2500 \times g$) to separate porewater from sediments. Porewaters were filtered (Target GL Microfiber 0.7 μm ; Thermo Scientific National, Rockwood, Tennessee, USA) and preserved with analytical-grade nitric acid, whereas solids were

frozen (-20°C) and then freeze-dried. Porewater and solid samples were analyzed for a suite of elements by inductively coupled plasma mass spectrometry on a Perkin Elmer Elan6000 quadrupole ICP-MS (Perkin Elmer, Waltham, Massachusetts, USA). Solid samples were extracted with analytical grade nitric acid prior to ICP-MS analysis. In the intensive mesocosms, high-resolution profiles of sulfide were measured using a H₂S₅₀ microelectrode (Unisense A/S, Aarhus, Denmark), and surface sediments were examined using X-ray diffraction and element mapping as described in Orihel et al. (2015).

Water and phytoplankton sampling

Water was collected from the 15 mesocosms and an adjacent lake reference site every 2 weeks from June to October 2009 and every month from January to March 2010. The top 4 m of the water column was sampled using an acid-cleaned polyvinyl chloride tube fitted with a foot valve (3/4" inner diameter; 1 inch = 2.56 cm). Each water sample was subsampled for nutrient, phytoplankton, and microcystin and analyzed as described in Orihel et al. (2015). Nutrient (P and N) determinations were performed on unfiltered water for “total” concentrations and on filtered water (0.7- μm Whatman GF/F filters; Sigma Aldrich, St. Louis, Missouri, USA) for “dissolved” concentrations. In this study, dissolved N and P refer to total dissolved nutrient concentrations. Phytoplankton were concentrated on GF/F filters under low light and kept frozen (-80°C) until analysis for pigments using standard reversed-phase high performance liquid chromatography. Samples for taxonomic enumeration of phytoplankton were fixed with Lugol’s solution and formaldehyde-acetic acid and enumerated using light microscopy following the Utermöhl technique. Microcystin samples were stored frozen (-20°C) and in darkness, and analyzed by protein phosphatase inhibition assays.

Periphyton sampling

Four strips of mesocosm wall material (10 \times 3 m) were hung vertically from wooden floats in each mesocosm at the beginning of the experiment, and harvested monthly to estimate P accumulation and algal biomass on mesocosm walls. Harvested strips were scraped clean, diluted with MilliQ water to a volume of 1 L, homogenized with a blender, and subsampled for total P and chlorophyll *a* (chl *a*).

Phosphorus budgets

A daily phosphorus (P) budget was constructed for each mesocosm based on the following mass balance equation:

$$\Delta W_t = (A_t + I_t) - (S_t + \Delta P_t)$$

where ΔW_t = change in P mass in the water column from day *t* to day *t* + 1, A_t = P flux from atmospheric

deposition on day t , I_t = P flux from internal loading on day t , S_t = P flux from sedimentation on day t , and ΔP_t = change in P mass in periphyton growing on mesocosm walls on day t . As described in Appendix S2, the parameters ΔW_t , A_t , S_t , and ΔP_t were estimated on a daily basis from Julian day 162 to 294 (11 June–21 October 2009) in order to solve for the unknown parameter, I_t . For each parameter, a cumulative sum was calculated over the entire period which was then divided by the cross-sectional surface area of the mesocosm and the number of days in the period to express an average daily flux rate in $\text{mg P}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$; for example, internal P loading was estimated as $[\sum_{t=162}^{294} (I_t + I_{t+1})]/3.14/132$.

Statistical analyses

Statistical analyses were performed using SigmaPlot for Windows (Version 12.5, Systat Software, Inc., San Jose, California, USA). We examined how nutrient concentrations in sediment and water, growth of phytoplankton and periphyton, and microcystin concentrations responded as a function of Fe dose applied to mesocosms. Dose–response relationships among Fe-treated mesocosms ($n = 12$) were fitted using linear regression models and tested using analysis of variance. Normality and equal variance tests were used to confirm assumptions of parametric tests, and data was \log_{10} -transformed as required. In addition, we performed one-sample t tests to assess whether the mean of control mesocosms ($n = 3$) is significantly different from either: (1) the intercept of the dose–response relationship among Fe-treated mesocosms (if this relationship was significant) or (2) the mean of Fe-treatment mesocosms (if the dose–response relationship was not significant). For all statistical analyses, we used an alpha value of 0.05.

RESULTS

Sediment chemistry

Addition of ferric chloride (2–225 g Fe/ m^2 sediment) to a series of mesocosms generated a gradient of Fe in surface sediments from background levels to a maximum of 94 mg/g dry mass in the solid phase and 6 mg/L in porewater. Iron concentrations in the 12 treated mesocosms increased significantly as a function of Fe dose in both sediment and porewater (Fig. 2a,b; but note in Fig. 2a that the mean of the three control mesocosms was significantly higher than the intercept of the dose–response relationship; t test, $t_2 = 48$, $P = 0.0004$). Sediment P was similar among all mesocosms (~ 2 mg/g dry mass; Fig. 2c), but Fe addition strongly decreased dissolved P in porewater (Fig. 2d). Porewater Fe and P were also affected by Fe addition in deeper sediment layers (Fig. 2e,f). By mass, the Fe:P ratio in near-surface porewater of controls was 0.1–0.4, whereas all mesocosms receiving at least 18 g Fe/ m^2 had Fe:P ratios greater than 2.

Iron suppressed the build-up of H_2S in porewaters (Fig. 2g) as a result of the precipitation of Fe sulfides.

A black solid was visible on the surface of Fe-amended sediments, and the examination of minerals using X-ray diffraction confirmed the presence of pyrite (Fig. S2). The Fe-bearing hydroxide goethite [$\text{Fe}^{+3}\text{O}(\text{OH})$] and the phosphate mineral apatite [$\text{Ca}_{10}(\text{V}_{0.9}\text{P}_{0.1}\text{O}_4)_6\text{F}_2$] were observed in some mesocosms amended with Fe (Fig. S2). Element mapping showed Fe–Ca–P mineral phases in Fe-treated sediments that were absent in control sediments (Fig. S3). Other common minerals observed were quartz, calcite, dolomite, albite, orthoclase, montmorillonite, kaolinite, and illite.

Water column nutrients

In the lake and control mesocosms, a large rise in total P (>100 $\mu\text{g/L}$; Fig. 3a) and dissolved P (>25 $\mu\text{g/L}$; Fig. S4a) was observed in late summer/early fall, and also under ice in winter. Similar accumulations of P were observed in mesocosms receiving low doses of Fe, but were increasingly suppressed at higher doses (Fig. 3b–d; Fig. S4b–d). Among the Fe-treated mesocosms, the negative effect of Fe on P was strongly dose-dependent during the open-water season and in winter for both total P (Fig. 4a,b) and dissolved P (Fig. S5a,b), with Fe treatment decreasing average concentrations by up to 56–72% (relative to the mean of controls). Total N (Fig. 3e–h) and dissolved N (Fig. S4e–h) were depressed immediately following Fe treatment, but this effect was temporary. Iron significantly lowered total and dissolved N in the open-water season (Fig. 4c; Fig. S5c) but not in winter (Fig. 4d; Fig. S5d). Mean concentrations of N and P in the control mesocosms were not significantly different from the intercepts of these dose–response relationships (t tests, $P > 0.05$), with the exception of dissolved N during the open-water season (t test, $t_2 = -8$, $P = 0.01$; Fig. S5c).

Total N:P ratios in the lake and controls decreased steadily by two- to threefold over the summer and to a lesser extent (25–30%) in winter; in contrast, total N:P ratios in most Fe-treated mesocosms showed transient increases in early summer, and in the case of high-Fe mesocosms, little change under ice (Fig. 3i–l). Iron-treated mesocosms began the open-water season at higher dissolved N:P ratios than controls, but all showed a decline over time (Fig. S4i–l). Overall, Fe treatment significantly increased average ratios of total N:P (Fig. 4e,f) and dissolved N:P (Fig. S5e,f) during the open-water season and in winter.

Phytoplankton

Iron suppressed total phytoplankton biomass, as inferred from changes in chl a (Fig. 5a–d) and beta carotene (Fig. S6a–d). Temporal trends in these pigments in the lake and controls showed an algal bloom occurred in August, and a second, smaller bloom in late September. Blooms of equal or greater magnitude also occurred in low-Fe mesocosms, but were subdued by higher Fe doses. Among the Fe-treated mesocosms, average chl a

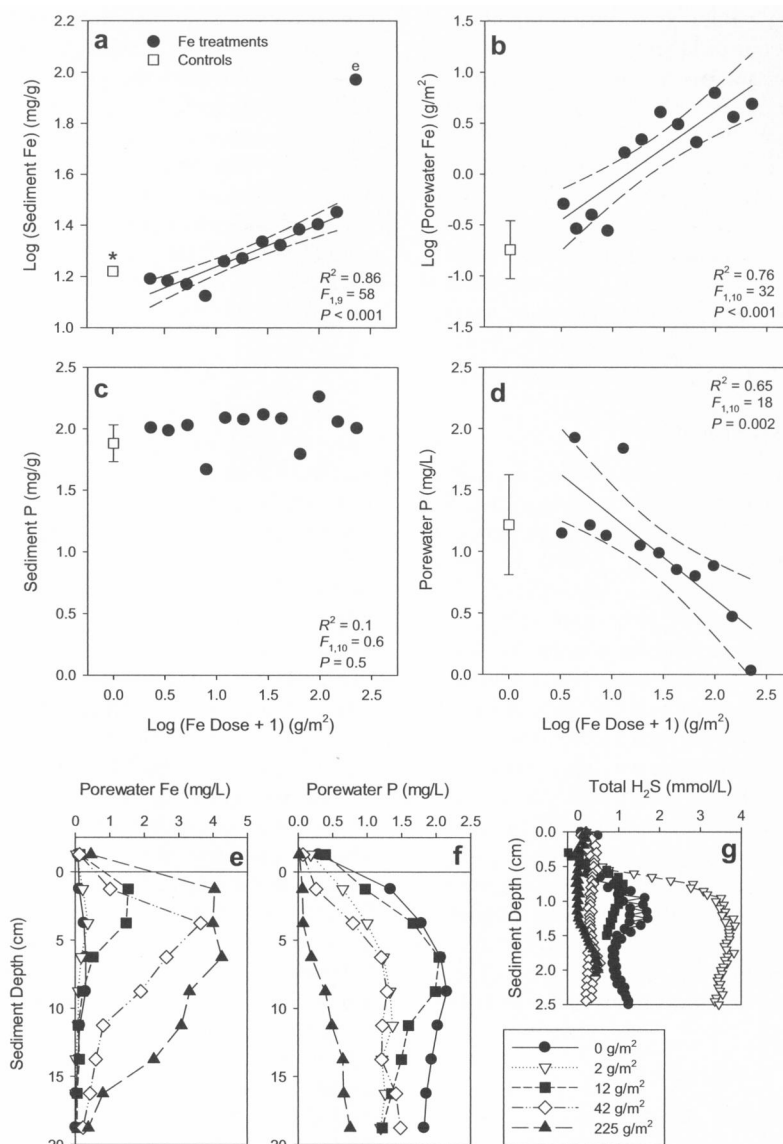


FIG. 2. Chemistry of sediments in iron-treated and control mesocosms at the end of the experiment. Iron and phosphorus concentrations in the (a, c) solid phase and (b, d) porewater of surface (0–2.5 cm) sediments, shown as dry mass. In panels a–d, the fitted regression line (solid line) and 95% confidence intervals (dashed lines) of the data from the iron-treated mesocosms are shown along with associated test statistics. The “e” indicates an outlier that was excluded from the model. Values for control mesocosms ($n = 3$) are shown as mean \pm standard error [SE], with an asterisk indicating the mean of the controls is significantly ($P < 0.05$) different from the iron-treated mesocosms (see text under heading “Statistical analyses”). Vertical concentration profiles of (e) porewater iron, (f) phosphorus, and (g) total sulfide in sediments of five intensively sampled mesocosms.

over the open-water season significantly declined as a function of the dose of Fe applied (Fig. 6a). Likewise, average beta carotene over the same period was strongly reduced by Fe ($R^2 = 0.58$, $F_{1,10} = 14$, $P = 0.004$). The visual difference in algal growth between control and Fe-treated mesocosms was striking (Fig. 1d,e). Photosynthetic bacteria, as indicated by bacterial chlorophyll (Fig. 5e–h), also significantly decreased as a function of Fe dose (Fig. 6b). Although chl *a*, beta carotene, and bacterial chlorophyll in controls tended to be lower than expected, control means had large standard

errors and were not significantly different from intercepts of dose–response relationships (Fig. 6a,b; *t* tests, $P > 0.05$).

Pigments representative of all cyanobacteria (zeaxanthin; Fig. 5i–l), filamentous cyanobacteria (canthaxanthin; Fig. 5m–p), and colonial cyanobacteria (myxoxanthophyll; Fig. 5q–t) responded to Fe additions: their average concentrations during the open-water season were significantly negatively related to Fe dose among treated mesocosms (Fig. 6c–e). Concentrations of these pigments in controls were again lower than expected

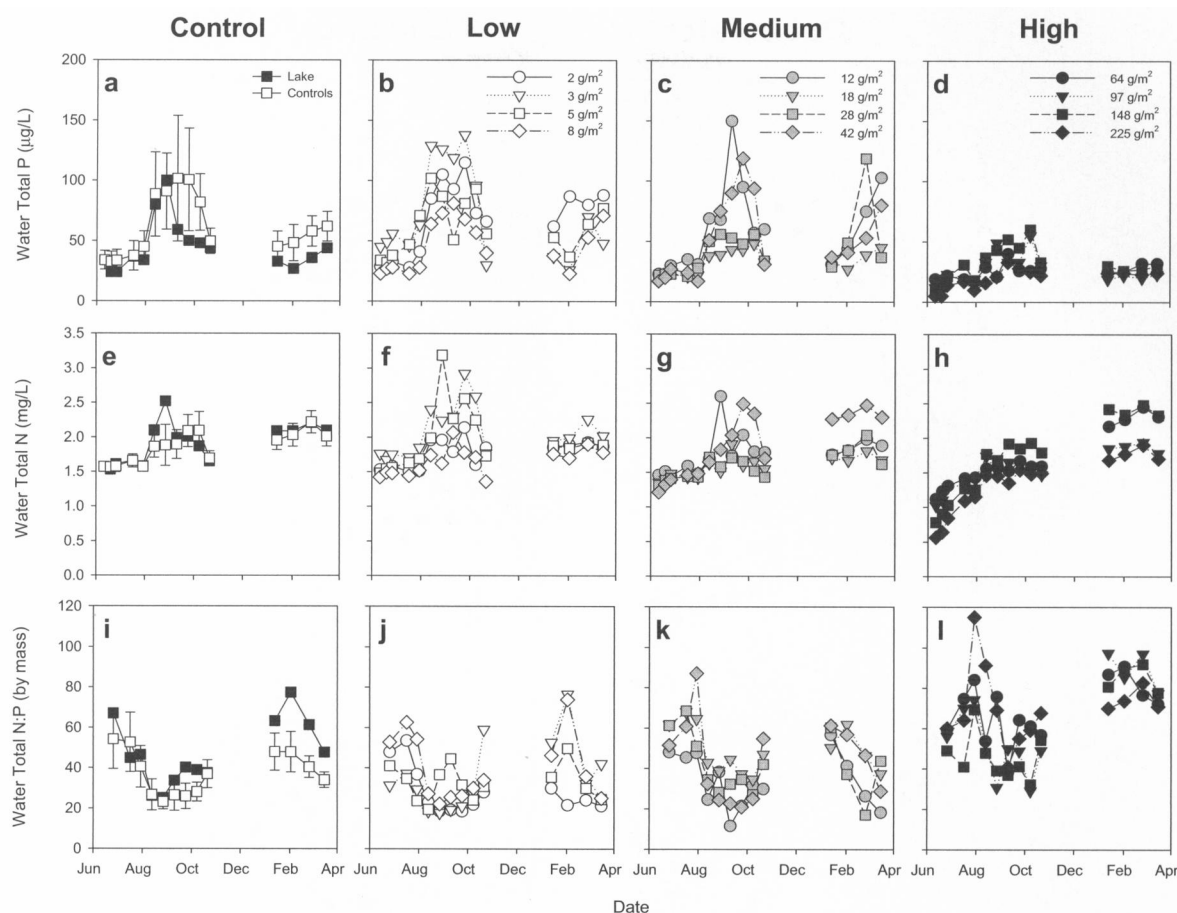


FIG. 3. Concentrations of the important algal nutrients phosphorus and nitrogen in Nakamun Lake, control mesocosms (mean \pm SE, $n = 3$), and 12 mesocosms treated with different doses of iron (2–225 g Fe/m² sediment). (a–d) Total phosphorus, (e–h) total nitrogen, and (i–l) total nitrogen to total phosphorus ratios in the water column from June to October, and under ice in January to March.

(Fig. 6c–e), but only in the case of zeaxanthin was the mean significantly lower than the intercept of the dose–response relationship (t test, $t_2 = -5$, $P = 0.04$). In contrast, pigments indicative of chlorophytes (chlorophyll *b*; Fig. S6e–h), cryptophytes (alloxanthin; Fig. S6i–l), and chrysophytes/diatoms/dinoflagellates (fucoxanthin and diadinoxanthin; Fig. S6m–t) were not significantly related to Fe dose (chlorophyll *b*, $R^2 = 0.07$, $F_{1,10} = 1$, $P = 0.4$; alloxanthin, $R^2 = 0.14$, $F_{1,10} = 2$, $P = 0.2$; fucoxanthin, $R^2 = 0.28$, $F_{1,10} = 4$, $P = 0.08$; and diadinoxanthin, $R^2 = 0.04$, $F_{1,10} = 0.4$, $P = 0.5$). Mean concentrations of these pigments in controls were not significantly different from those of Fe-treated mesocosms (t tests, $P > 0.05$).

Iron additions also altered phytoplankton community composition based on taxonomic cell enumerations performed at the end of July, August, and September (Fig. 7a–c). Low-Fe mesocosms contained mainly cyanobacteria in summer and diatoms/chlorophytes in the fall, whereas chrysophytes and mixed algal assemblages were more common in mesocosms receiving higher Fe doses. Phytoplankton in the study lake and controls first

contained mixed assemblages that were later succeeded by cyanobacteria. Importantly, average biomass of cyanobacteria was significantly reduced as a function of Fe dose (Fig. S7a). Higher iron also significantly reduced diatoms while stimulating chrysophytes (Fig. S7b–c), but had no significant effect on other algal groups (euglenophytes, $R^2 = 0.3$, $F_{1,10} = 5$, $P = 0.05$; cryptophytes, $R^2 = 0.2$, $F_{1,10} = 2$, $P = 0.2$; chlorophytes, $R^2 = 0.2$, $F_{1,10} = 3$, $P = 0.1$; and dinoflagellates, $R^2 = 0.0001$, $F_{1,10} = 0.002$, $P = 1$).

On average, cyanobacterial biomass as a percentage of total phytoplankton biomass was quite variable, but significantly negatively related to Fe dose (Fig. 7d). This relationship was due to the suppression of non-heterocystous, rather than heterocystous, cyanobacteria (Fig. 7e–f). On the other hand, relationships between Fe dose and pigment-based indicators of cyanobacterial dominance were not significant (zeaxanthin: chl *a*, $R^2 = 0.09$, $F_{1,10} = 1$, $P = 0.3$; canthaxanthin, chl *a*: $R^2 = 0.31$, $F_{1,10} = 4$, $P = 0.06$; and myxoxanthophyll, chl *a*: $R^2 = 0.12$, $F_{1,10} = 1$, $P = 0.3$).

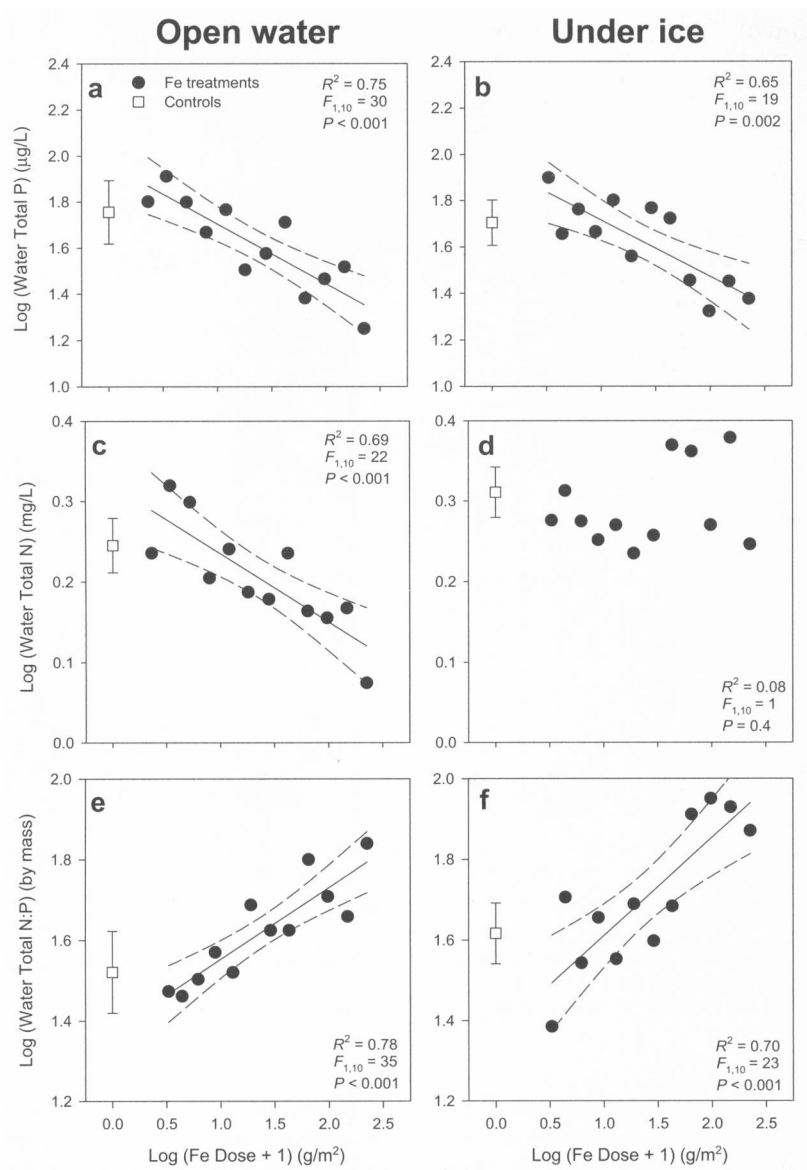


FIG. 4. Relationships between iron dose applied to mesocosms and average concentrations of (a, b) total phosphorus, (c, d) total nitrogen, and (e, f) ratios of total nitrogen to total phosphorus. Each point represent the mean of measurements in a mesocosm from June to October ($n = 11$; panels a, c, and e), or from January to March ($n = 3$; panels b, d, and f). Statistics are as in Fig. 2.

Microcystin

Microcystin concentrations in most Fe-treated mesocosms remained below the 1 µg/L drinking water guideline set by the World Health Organization (WHO), whereas levels in the lake and controls were above the WHO guideline in late summer and fall (Fig. 5u–x). Microcystin in the controls reached as high as 5.5 µg/L, with average concentrations over the open-water season ranging from 0.6 to 2.4 µg/L. In comparison, average concentrations in Fe-treated mesocosms over this same period did not exceed 0.6 µg/L, with the exception of the mesocosm treated with 42 g Fe/m². Overall, there was no relationship between Fe dose and microcystin among treated

mesocosms (Fig. 6f). The mean of the controls was higher than most treated mesocosms (Fig. 6f), although this difference was not statistically significantly (t test, $t_2 = 4$, $P = 0.14$).

Periphyton

Periphytic growth on the inner mesocosm walls was inhibited at high doses of Fe. Phosphorus accumulated in periphyton slowly over summer, and more rapidly in fall, with the highest accumulation in low-Fe mesocosms (Fig. 8a–d). Likewise, periphytic chl *a* increased in concentration over time, but less growth was observed in mesocosms receiving more Fe (Fig. 8e–h). On average,

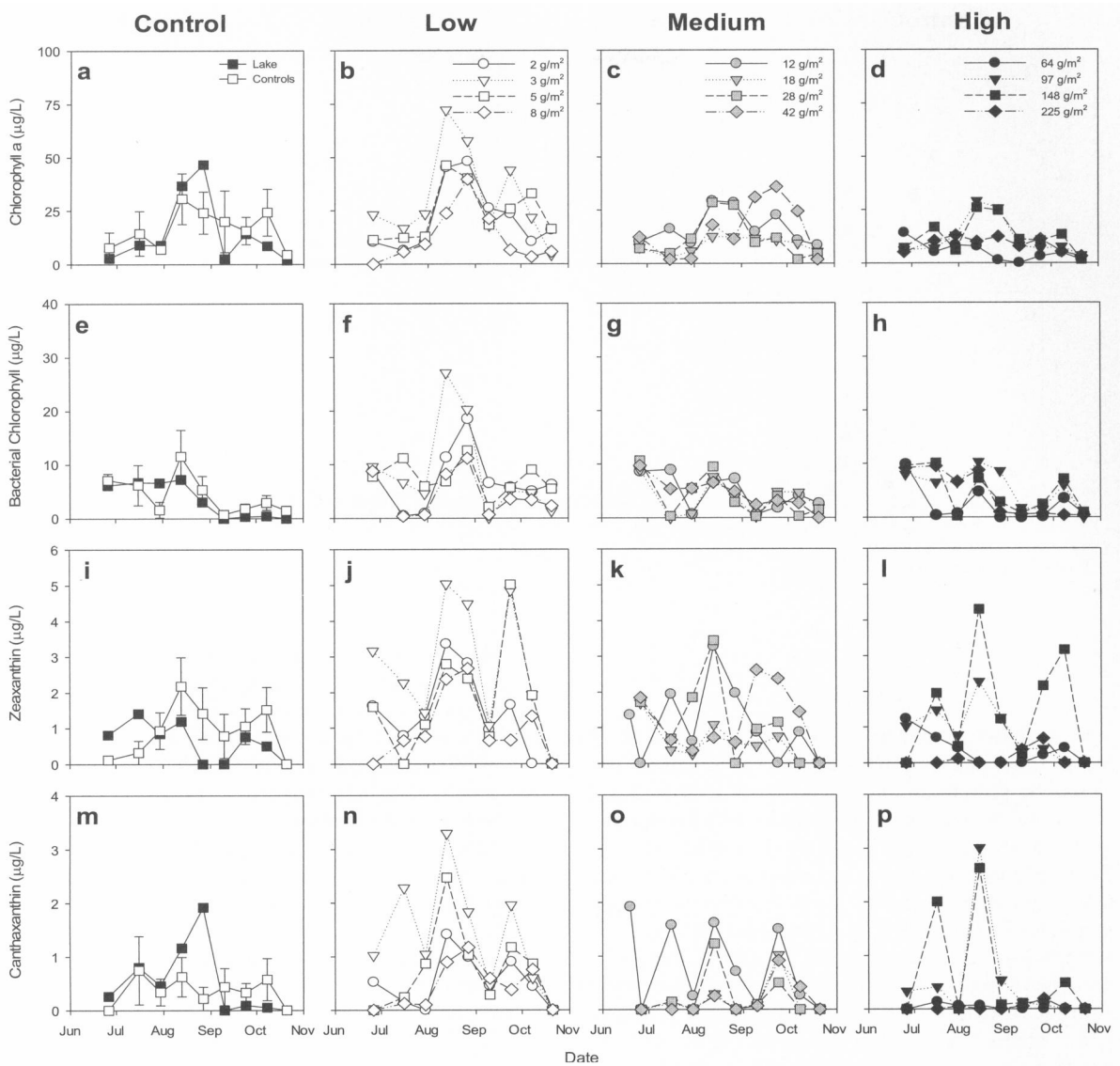


FIG. 5. Concentrations of photosynthetic pigments in Nakamun Lake, control mesocosms (mean \pm SE, $n = 3$), and 12 mesocosms treated with different doses of iron. Pigments are indicators of the biomass of various groups: (a–d) chlorophyll *a* is produced by all algae; (e–h) bacterial chlorophyll is produced by photosynthetic bacteria; (i–l) zeaxanthin is produced by all cyanobacteria; and (m–p) canthaxanthin and (q–t) myxoxanthophyll are produced by filamentous and colonial cyanobacteria, respectively. Concentrations of the (u–x) hepatotoxin microcystin, produced by certain cyanobacteria, are shown with the dashed line indicating the World Health Organization’s drinking water guideline for microcystin-LR (Microcystin-LR is one of the isoforms of microcystin. The WHO guideline specifically refers to microcystin-LR).

total P and chl *a* were significantly inversely related to Fe dose, with treatment explaining 77% and 82% of the variation in total P and chl *a*, respectively (Fig. 9).

Phosphorus budgets

Internal loading of P from sediments was the major input of P to the water column of mesocosms from June to October (Table 2), with Fe significantly decreasing the P flux from sediments by up to two- to threefold ($R^2 = 0.59$, $F_{1,10} = 14$, $P = 0.004$). On average, the daily

rate of internal P loading ranged from 8 mg·m⁻²·d⁻¹ in the mesocosm receiving the highest Fe dose to over 30 mg·m⁻²·d⁻¹ in a control mesocosm. In contrast to internal loading, precipitation added negligible amounts of P to mesocosms over the same period. Sedimentation was the major mechanism of P loss from the water column, whereas uptake of P by periphyton was a relatively minor flux. In winter, sediments in all mesocosms released much less P than in summer, and no internal P loading was measurable in the mesocosms receiving the two highest doses (Table 2).

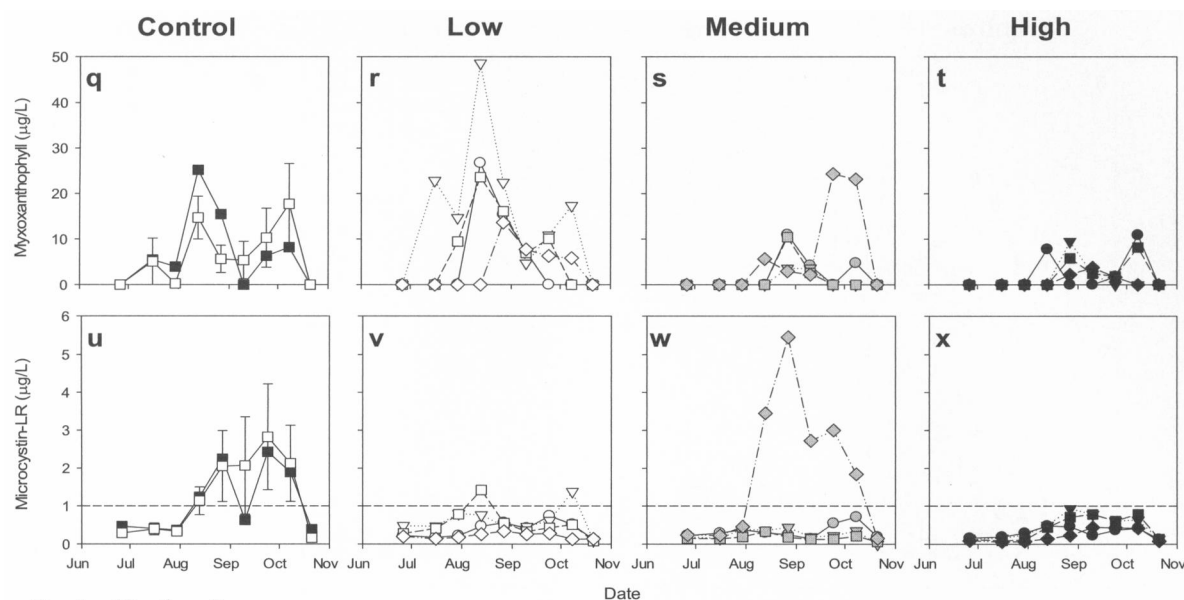


FIG. 5. (Continued)

DISCUSSION

Hypothesis 1

Consistent with our hypothesis, increasing Fe in sediments lowered concentrations of P in sediment porewaters by preventing an accumulation of H_2S (Fig. 2). Our study lake was an ideal system to test this hypothesis as it is moderately high in sulfate (13 mg/L) and low in Fe in sediments (16 mg/g) and porewaters (<0.5 mg/L). Our empirical evidence of lower P in porewaters with increasing Fe concentration is consistent with the inverse relationship between porewater Fe and P observed by Smolders and Roelofs (1993) across a range of sediments. Amended Fe appeared to buffer against H_2S accumulation as reported elsewhere (Giordani et al. 1996, Van der Welle et al. 2007, Zhu et al. 2012), which is noteworthy given previous evidence of sulfate loading mobilizing P from sediments (Caraco et al. 1989, 1993, Smolders et al. 2006). This may occur by reduction of Fe(III) oxides or Fe(II) minerals by H_2S produced through microbial sulfate reduction, as well as depletion of porewater Fe by precipitation of FeS_x (Roden and Edmonds 1997, Gächter and Müller 2003, Katsev et al. 2006).

Results of X-ray diffraction revealed the presence of goethite in Fe-treated sediments (Fig. S2d,e), thus sorption of P onto Fe(III) oxides may have contributed to limiting P diffusion across the sediment–water surface. Nanophase goethite has previously been identified as the primary reactive Fe oxyhydroxide phase in lacustrine and marine environments (Van der Zee et al. 2003). In some sediments, a substantial amount of P in sediments may be sequestered in amorphous Fe(III) phases (Rozan et al. 2002). Although we expected that Fe treatment would result in formation of Fe(II) phosphate minerals, we were

not able to show conclusively that these minerals were formed in sediments. Element mapping of Fe-treated sediments by electron microprobe suggested the formation of a mineral containing Fe, P, and Ca (Fig. S3), but the only P-bearing mineral identified by X-ray diffraction was apatite (Fig. S2). However, the direct identification of Fe(II) phosphate minerals, such as vivianite, is technically challenging, as demonstrated by a recent study which concluded that vivianite cannot be identified by X-ray diffraction of bulk sediment samples (Rothe et al. 2014). By applying a heavy-liquid separation to sediments, Rothe et al. (2014) confirmed extensive vivianite formation in sediments of a German lake following Fe treatment.

Hypothesis 2

Our findings also supported the hypothesis that Fe reduced the net internal P load from sediments during the open-water season in a dose-dependent manner (Table 2). In Nakamun Lake, internal loading contributes substantially to the whole-lake P budget (Riley and Prepas 1984) and can cause the mass of P in the water column to double from spring to fall (Orihel et al. 2015). Based on mass balances of control mesocosms (Table 2), we estimated that the average daily rate of P release from sediments from June to October was $19 \text{ mg} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$, which is comparable to previous estimates of internal P loading for this lake ($10\text{--}20 \text{ mg} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$; Riley and Prepas 1984), and those of other hypereutrophic lakes (average of $22 \text{ mg} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$ for 30 lakes; Carter and Dzialowski 2012). Importantly, Fe amendment reduced sediment P flux down to $8 \text{ mg} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$ at the highest dose. This Fe-mediated suppression of internal loading significantly lowered P

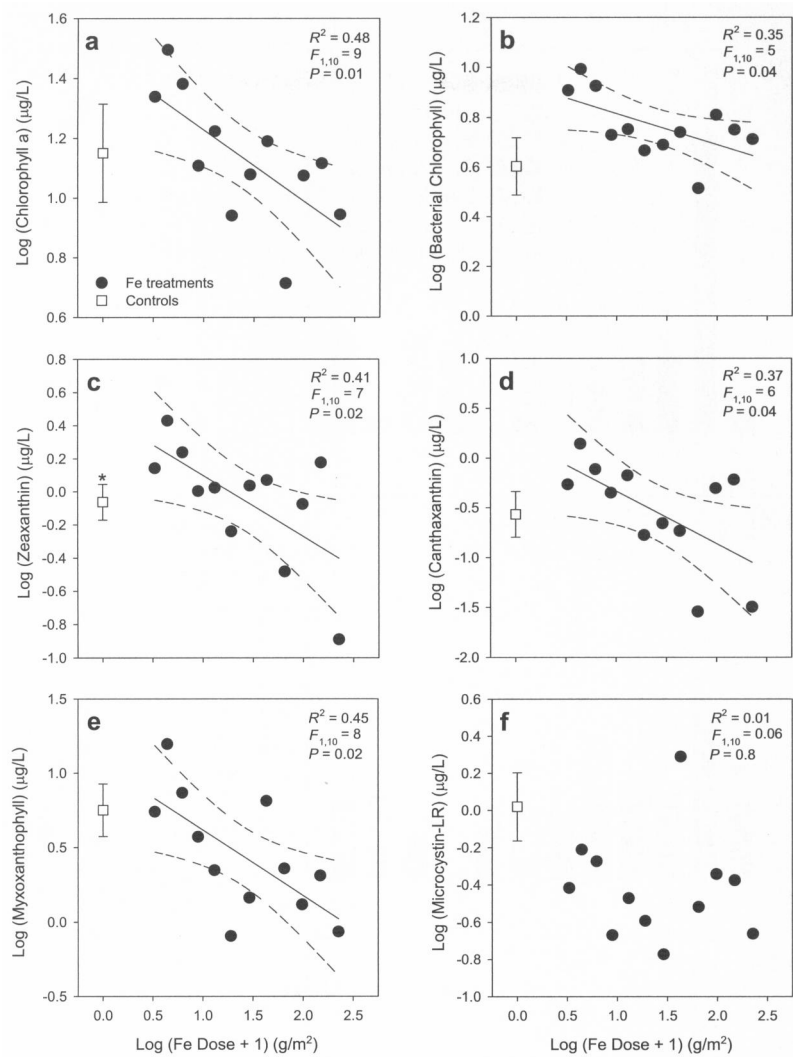


FIG. 6. Relationships between iron dose applied to mesocosms and average ($n = 9$) concentrations of the algal pigments (a) chlorophyll *a*, (b) bacterial chlorophyll, (c) zeaxanthin, (d) canthaxanthin, (e) myxoxanthophyll and the (f) cyanobacterial toxin microcystin. Statistics are as in Fig. 2, pigment descriptions as in Fig. 5.

concentrations in the water column (Fig. 4; Fig. S5), diminishing the large peak in P that typically occurs in late summer, as well as reducing the accumulation of P under ice in winter (Fig. 3; Fig. S4).

Based on our previous research in Nakamun Lake (Orihel et al. 2015), we expected the nutrient flux from Fe-enriched sediments to be characterized by higher N:P ratios than Fe-deficient sediments. The relative amount of N to P in freshwater lakes is an important metric, as the ratio of these elements may influence phytoplankton community composition (Smith and Bennett 1999). Consistent with this prediction, strong positive relationships between Fe dose and total N:P ratio (Fig. 4), as well as dissolved N:P ratio (Fig. S5) were observed. In agreement with our study, an increase in total N:P was reported for Lac Heney in response to Fe treatment (Carignan 2014).

Hypothesis 3

Our results strongly supported the hypothesis that suppression of water column P concentrations by Fe addition would lower algal growth. Among Fe-treated mesocosms, both phytoplankton and periphyton biomass (inferred based on chl *a*) declined significantly with increasing Fe dose (Figs. 6 and 9). We observed that blooms of phytoplankton in summer (Fig. 5) and of periphyton in fall (Fig. 8) occurred at lower Fe doses (which had a negligible impact on P cycling), but were diminished in mesocosms that had received higher Fe doses. Algal blooms in mesocosms receiving the lowest Fe doses were similar, if not higher, than those in control mesocosms, and therefore we cannot refute the hypothesis that Fe may stimulate phytoplankton growth in some circumstances.

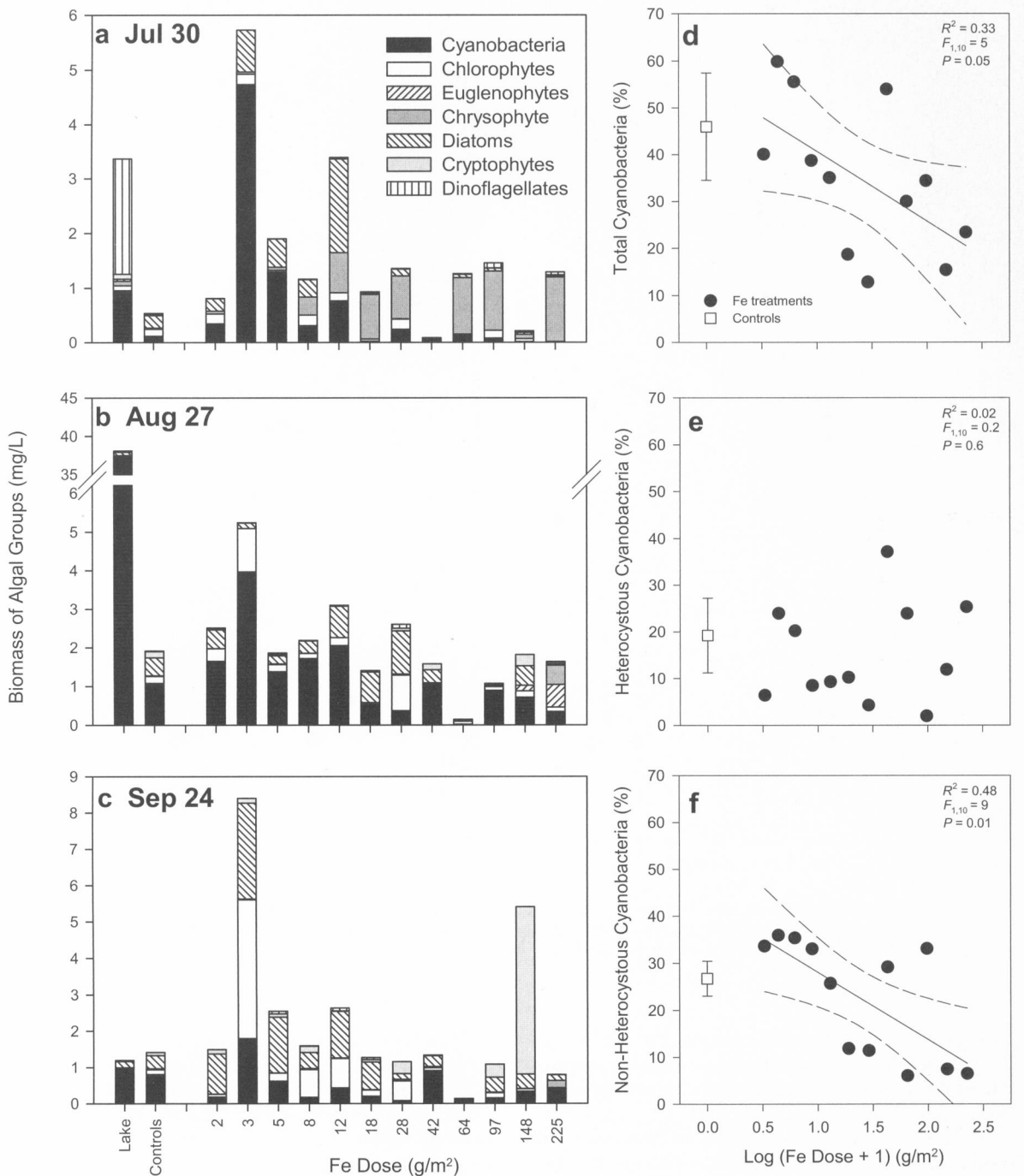


FIG. 7. Biomass of major phytoplankton groups in the lake, controls (average of $n = 3$), and iron-treated mesocosms on (a–c) three dates phytoplankton were enumerated, and relationships between iron dose applied to mesocosms and average ($n = 3$) (d) biomass of cyanobacteria, (e) heterocystous cyanobacteria, and (f) non-heterocystous cyanobacteria, relative to total phytoplankton biomass. Statistics are as in Fig. 2.

In agreement with our study, reductions in algal biomass have been observed in some lakes in response to Fe treatment (e.g., Lake Groß-Glienicke; Wolter 2010). Unfortunately, this has not always been the case due to continued high inputs of nutrients from external sources (Hayes et al. 1984, Boers et al. 1994) or an

insufficient dosage of Fe to inactivate P in sediments (note the three orders-of-magnitude range in Fe application rates in Table 1). The present study emphasizes that the success of Fe treatment as an in-lake remediation strategy is very much a function of the dose applied.

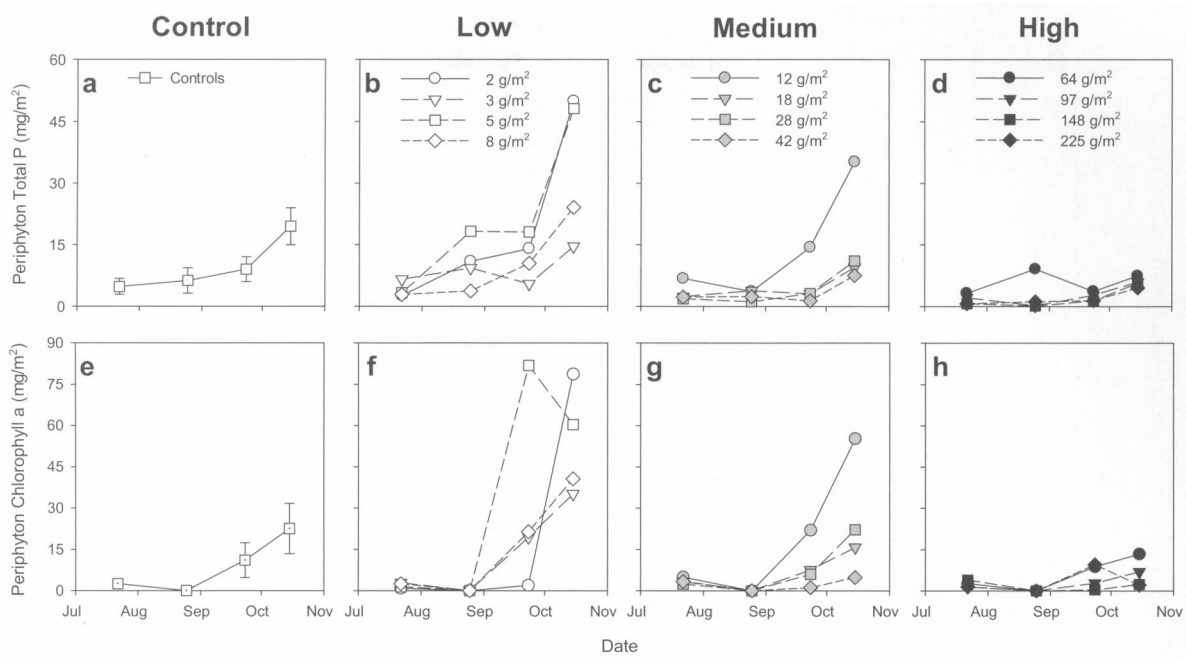


FIG. 8. Concentrations of total phosphorus (a–d) and chlorophyll *a* (e–h) in periphyton growing on the walls of control mesocosms (mean ± SE, *n* = 3) and 12 mesocosms treated with iron.

Experimental additions of Fe in Nakamun Lake not only lowered the biomass of algae, but also suppressed cyanobacterial blooms in a dose-dependent manner as inferred from both pigment concentrations (Fig. 6) and cell counts (Fig. 7). However, we had predicted diazotrophic cyanobacteria would decrease in Fe-amended mesocosms based on the expected increase in the ratio of N:P in the water column, but this did not occur. Our prediction was based on the hypothesis that a low N:P ratio favors species of cyanobacteria that fix atmospheric N₂, as this capacity gives them a competitive advantage over algae that rely solely on the pool of dissolved inorganic N (Schindler et al. 2008 and references therein). We did indeed observe that cyanobacteria became less dominant in the phytoplankton community with increasing Fe dose, but this was due to a reduction in the relative biomass of non-heterocystous, rather than heterocystous, cyanobacteria (Fig. 7). Although Fe treatment significantly increased N:P ratio (Fig. 4), this did not prevent the need for N₂-fixation (as heterocysts were observed in all mesocosms) and therefore, diazotrophy was still advantageous in high-Fe mesocosms.

Hypothesis 4

An interesting finding of our study was that Fe may suppress microcystin beyond its direct effect on cyanobacterial biomass. We hypothesized that higher levels of sediment Fe would decrease microcystin by reducing the P supply to, and thus the growth of, toxin-producing cyanobacteria. However, even low doses of Fe usually

kept microcystin concentrations below water quality guidelines, irrespective of high cyanobacterial biomass (Fig. 5). This is compelling in light of the discovery that Fe starvation triggers the transcription of microcystin synthetase genes and production of this toxin in the cyanobacterium *Microcystis* (Sevilla et al. 2008). Our results are in keeping with earlier work by Lukac and Aegerter (1993) that demonstrated *Microcystis* produced 20–40% more toxin under low (≤2.5 μmol/L) Fe conditions. Likewise, *Microcystis* under severe Fe stress (0.01 μmol/L) had significantly higher cellular microcystin concentrations than when cultured with 0.1 or 1 μmol/L of Fe (Alexova et al. 2011). However, other studies have reported that higher Fe levels (in the range of 5–16 μmol/L) stimulate microcystin production (Utkilen and Gjørlme 1995, Jiang et al. 2008, Li et al. 2009). Evidently the role of Fe in regulating microcystin requires further research.

An alternative explanation for the unexpectedly low microcystin levels in most Fe-treated mesocosms is that Fe could have played a role in the abiotic degradation of these toxins. Microcystins were rapidly broken down to nontoxic degradation products when exposed to 100 μg/mL FeCl₃ across a wide range of pH values by Takenaka and Tanaka (1995). More recent work determined that microcystin-LR forms complexes with Fe(III) (Klein et al. 2013) and adsorbs to micro- and nano-scale iron oxides (Gao et al. 2012). In fact, Fe-containing materials are currently being investigated for potential use in water treatment technologies to remove or detoxify microcystins (Dai et al. 2012, Marsalek et al. 2012, Fang et al. 2014).

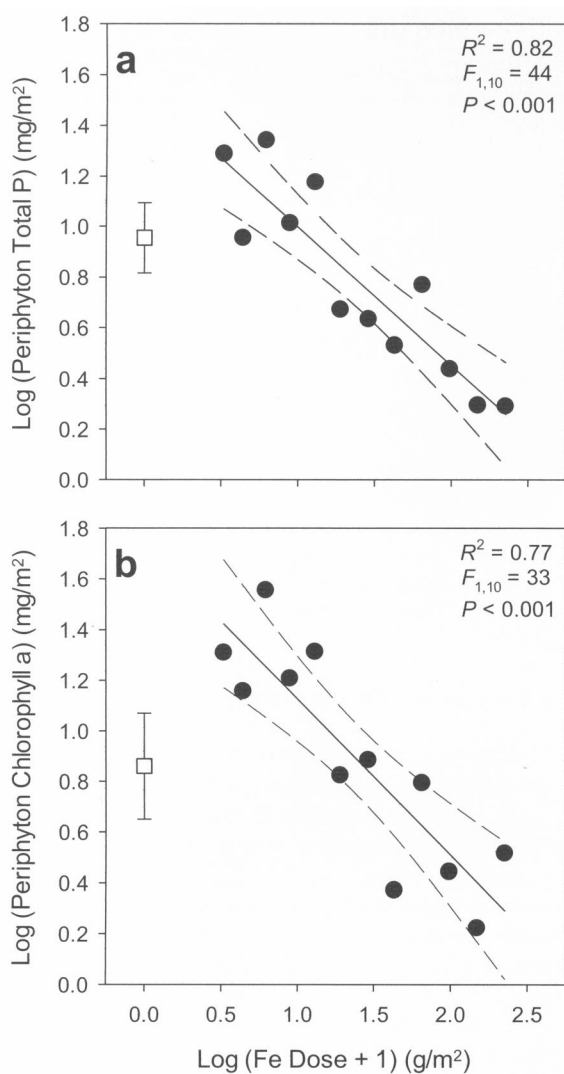


FIG. 9. Relationships between iron dose applied to mesocosms and average concentrations of (a) total phosphorus and (b) chlorophyll *a* in periphyton. Each point represent the mean of measurements in a mesocosm from July to October ($n = 4$). Statistics are as in Fig. 2.

Implications

Our study demonstrated that Fe amendment to a hypereutrophic lake sequestered P in sediments, which decreased P concentrations and increased N:P ratios in the water column. This in turn reduced the biomass of phytoplankton and periphyton, as well as shifted the composition of the phytoplankton from one largely dominated by cyanobacteria to a more diverse assemblage. Iron also seemed to reduce microcystin concentrations, not only by reducing cyanobacterial biomass, but by some other mechanism, potentially related to the inhibition of microcystin synthetase gene transcription. These substantial changes in nutrient cycling, algal biomass and species composition, and microcystin concentrations occurred in response to rather modest changes in

sediment Fe. The porewater Fe concentrations achieved even at the highest Fe dose are still lower than other lakes in the region connected to groundwater Fe sources (Ballard 2011) and an order of magnitude lower than some Canadian Shield lakes (e.g., Couture et al. 2010).

Our study suggests that Fe treatment is potentially an effective strategy for controlling HABs in eutrophic lakes with high internal P loading. The capacity of Fe to sequester P has led to the widespread use of Fe compounds in potable water and wastewater treatments, but only rarely in remediation of eutrophic lakes and reservoirs (Cooke et al. 2005). The primary use of Fe in these practices is usually as a coagulant; that is, to remove P from water by co-precipitation with Fe(III) oxyhydroxides. Because the mass of P in the water column of lakes is very small compared to the pool stored in sediments, the use of Fe simply to precipitate P in the water column has little value, and any effects are only temporary. Therefore, Fe treatment for the purposes of lake remediation should focus on inhibiting internal P loading from sediments by adding an appropriate dose to effectively sequester P in sediments.

Over the last 50 years, Fe salts have been applied to 16 lakes and reservoirs, ranging in size from 0.04 to 12 km², in nine countries (Table 1). These case studies demonstrate that Fe applications are logistically and financially feasible, at least in small- to medium-sized water bodies. The most compelling evidence that Fe treatment increases the long-term sequestration of P in sediments comes from Lake Vadnais, Minnesota, USA (Engstrom 2005) and Lake Groß-Glienicke, Germany (Kleeberg et al. 2012, 2013, Rothe et al. 2014). However, the success of Fe treatment as a remediation strategy is difficult to evaluate in many cases because studies are often confounded by the failure to control external sources of nutrients, absence of pre-treatment baseline data, insufficient Fe application doses, and/or simultaneous implementation of other remediation measures (e.g., food web biomanipulation, water column aeration, or other chemical sediment treatments). More carefully designed whole-lake studies are clearly needed to rigorously assess the longevity of Fe treatment. Notably, although the reduction of Fe under low redox conditions is often cited as the reason Fe treatments fail (e.g., Cooke et al. 1993), new research shows that the redox sensitivity of Fe does not impede lake restoration (Kleeberg et al. 2013). Finally, the use of zero-valent Fe nanoparticles (Li et al. 2006) for remediation of eutrophication would be an exciting avenue of future research.

Broadly speaking, our study points to the importance of Fe in controlling the trophic state of freshwater lakes, and raises intriguing questions about the sensitivity of certain lakes to changes in Fe supply in response to anthropogenic activities. Natural sources of Fe to lakes are typically dominated by fluvial inputs (Michard et al. 2001, Shaked et al. 2004, Maranger et al. 2006), although internal recycling from reduced sediments is substantial in some lakes (Campbell and Torgersen 1980, Nürnberg

TABLE 2. Flux of phosphorus into the water column of the mesocosms during the open water and winter seasons.

Fe dose (g/m ²)	Phosphorus flux into water column (mg·m ⁻² ·d ⁻¹)				
	Open water				Winter
	Rain	Sedimentation	Periphyton uptake	Internal loading	Internal loading
Control	0.1 (0.0)	-17.3 (5.9)	-1.3 (0.3)	19.0 (5.8)	1.2 (0.3)
2	0.1	-17.3	-3.5	22.0	1.9
3	0.1	-19.1	-0.8	19.2	0.8
5	0.1	-18.3	-3.3	22.2	1.7
8	0.1	-15.3	-1.7	17.5	2.4
12	0.1	-12.8	-2.5	16.4	5.2
18	0.1	-11.9	-0.6	12.8	0.8
28	0.1	-15.0	-0.8	16.0	0.6
42	0.1	-20.3	-0.4	21.1	3.1
64	0.1	-10.9	-0.5	11.6	0.5
97	0.1	-10.7	-0.4	11.6	0.3
148	0.1	-14.0	-0.4	15.2	0.0
225	0.1	-7.3	-0.3	8.1	0.0

Notes: Open-water season ran 11 June to 21 October 2009, winter season ran 13 January to 16 March 2010. Values in the control mesocosms (*n* = 3) column are shown with standard error in parens. Details of mass balance calculations are provided in Appendix S2.

and Dillon 1993) and seepage of Fe-rich groundwater may be important in others (Manning et al. 1991). Unfortunately, it is not known to what extent anthropogenic activities over the last century, such as the rise in global sulfur emissions (Stern 2005), have depleted natural stores of reactive Fe in lakes. Nor do we know how today’s growing dependence on sulfur fertilizers (Till 2010), disposal of massive amounts of sulfur wastes (Rappold and Lackner 2010), or changes in riverine discharges and groundwater supply due to climate change (Schindler and Donahue 2006), will affect the pool of available Fe in freshwater lakes in the future. In this study, we demonstrated how the delicate balance between Fe and S in a nutrient-rich, but Fe-poor, lake has far-reaching consequences for aquatic ecosystem health. Human activities that impact the natural cycling of Fe in lakes hold the potential to push some lakes over the delicate tipping point to a more eutrophic state, but scientific evidence to corroborate (or refute) this hypothesis is urgently needed.

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LITERATURE CITED

Alexova, R., M. Fujii, D. Birch, J. Cheng, T. D. Waite, B. C. Ferrari, and B. A. Neilan. 2011. Iron uptake and toxin synthesis in the bloom-forming *Microcystis aeruginosa* under iron limitation. *Environmental Microbiology* 13:1064–1077.

Ballard, N. C. 2011. Mechanisms and algal ecology of internal phosphorus loading in alkaline eutrophic lakes. Masters thesis. University of Alberta, Edmonton, Canada.

Boers, P., J. van der Does, M. Quaak, and J. van der Vlugt. 1994. Phosphorus fixation with iron(III)chloride: a new method to combat internal phosphorus loading in shallow lakes? *Archiv für Hydrobiologie* 129:339–351.

Boström, B., J. M. Andersen, S. Fleischer, and M. Jansson. 1988. Exchange of phosphorus across the sediment-water interface. *Hydrobiologia* 170:229–244.

Campbell, P., and T. Torgersen. 1980. Maintenance of iron meromixis by iron redeposition in a rapidly flushed monimolimnion. *Canadian Journal of Fisheries and Aquatic Sciences* 37:1303–1313.

Caraco, N. F., J. J. Cole, and G. E. Likens. 1989. Evidence for sulfate-controlled phosphorus release from sediments of aquatic systems. *Nature* 341:316–318.

Caraco, N. F., J. J. Cole, and G. E. Likens. 1993. Sulfate control of phosphorus availability in lakes. *Hydrobiologia* 253:275–280.

Carignan, R. 2014. Évolution de la qualité des eaux au lac Heney entre mars 2007 et mars 2014. Université de Montréal, Montréal, Canada.

Carmichael, W. 2008. A world overview – one-hundred-twenty-seven years of research on toxic cyanobacteria – where do we go from here? *Advances in Experimental Medicine and Biology* 619:105–125.

- Carter, L. D., and A. R. Dzialowski. 2012. Predicting sediment phosphorus release rates using landuse and water-quality data. *Freshwater Science* 31:1214–1222.
- Coale, K. H., et al. 1996. A massive phytoplankton bloom induced by an ecosystem-scale iron fertilization experiment in the equatorial Pacific Ocean. *Nature* 383:495–501.
- Cooke, G. D., E. B. Welch, A. B. Martin, D. G. Fulmer, J. B. Hyde, and G. D. Schriever. 1993. Effectiveness of Al, Ca, and Fe salts for control of internal phosphorus loading in shallow and deep lakes. *Hydrobiologia* 253:323–335.
- Cooke, G. D., E. B. Welch, S. A. Peterson, and S. A. Nichols. 2005. Restoration and management of lakes and reservoirs, Third edition. Taylor & Francis, CRC Press, Boca Raton, Florida, USA.
- Cornelis, P., and S. C. Andrews. 2010. Iron uptake and homeostasis in microorganisms. Caister Academic Press, Norfolk, UK.
- Couture, R.-M., C. Gobeil, and A. Tessier. 2010. Arsenic, iron and sulfur co-diagenesis in lake sediments. *Geochimica et Cosmochimica Acta* 74:1238–1255.
- Dai, G., C. Quan, X. Zhang, J. Liu, L. Song, and N. Gan. 2012. Fast removal of cyanobacterial toxin microcystin-LR by a low-cytotoxic microgel-Fe(III) complex. *Water Research* 46:1482–1489.
- Daldorph, P. W. G. 1999. A reservoir in management-induced transition between ecological states. *Hydrobiologia* 395/396:325–333.
- Deppe, T., and J. Benndorf. 2002. Phosphorus reduction in a shallow hypereutrophic reservoir by in-lake dosage of ferrous iron. *Water Research* 36:4525–4534.
- Deppe, T., K. Ockenfeld, A. Meybohm, M. Opitz, and J. Benndorf. 1999. Reduction of *Microcystis* blooms in a hypertrophic reservoir by a combined ecotechnological strategy. *Hydrobiologia* 408/409:31–38.
- Dokulil, M. T., K. Donabaum, and K. Pall. 2011. Successful restoration of a shallow lake: a case study based on bistable theory. Pages 285–294 in A. A. Ansari, S. S. Gill, G. R. Lanza and W. Rast, editors. *Eutrophication: causes, consequences, and control*. Springer, New York, New York, USA.
- Donabaum, K., M. Schagerl, and M. T. Dokulil. 1999. Integrated management to restore macrophyte domination. *Hydrobiologia* 395/396:87–97.
- Dove, A., and S. C. Chapra. 2015. Long-term trends of nutrients and trophic response variables for the Great Lakes. *Limnology and Oceanography* 60:696–721.
- Downs, T. M., M. Schallenberg, and C. W. Burns. 2008. Responses of lake phytoplankton to micronutrient enrichment: a study in two New Zealand lakes and an analysis of published data. *Aquatic Sciences* 70:347–360.
- Engstrom, D. R. 2005. Long-term changes in iron and phosphorus sedimentation in Vadnais Lake, Minnesota, resulting from ferric chloride addition and hypolimnetic aeration. *Lake and Reservoir Management* 21:95–105.
- Fagel, N., L. Y. Alleman, L. Granina, F. Hatert, E. Thamo-Bozso, R. Cloots, and L. André. 2005. Vivianite formation and distribution in Lake Baikal sediments. *Global and Planetary Change* 46:315–336.
- Fang, Y., Y. Zhang, W. Ma, D. M. Johnson, and Y. Huang. 2014. Degradation of microcystin-LR in water: hydrolysis of peptide bonds catalyzed by maghemite under visible light. *Applied Catalysis B: Environmental* 160–161:597–605.
- Foy, R. H. 1985. Phosphorus inactivation in a eutrophic lake by the direct addition of ferric aluminum sulfate: impact on iron and phosphorus. *Freshwater Biology* 15:613–629.
- Foy, R. H., and A. G. Fitzsimons. 1987. Phosphorus inactivation in a eutrophic lake by the direct addition of ferric aluminum sulfate: changes in phytoplankton populations. *Freshwater Biology* 17:1–13.
- Gächter, R., and B. Müller. 2003. Why the phosphorus retention of lakes does not necessarily depend on the oxygen supply to their sediment surface. *Limnology and Oceanography* 48:929–933.
- Gao, Y., N. Gao, Y. Deng, J. Gu, Y. Shen, and S. Wang. 2012. Adsorption of microcystin-LR from water with iron oxide nanoparticles. *Water Environment Research* 84:562–568.
- Giordani, G., M. Bartoli, M. Cattadori, and P. Viaroli. 1996. Sulphide release from anoxic sediments in relation to iron availability and organic matter recalcitrance and its effects on inorganic phosphorus recycling. *Hydrobiologia* 329:211–222.
- Goldyn, R., B. Messyas, P. Domek, W. Windhorst, C. Huguenschmidt, M. Nicoara, and G. Plavan. 2013. The response of Lake Durowskie ecosystem to restoration measures. *Carpathian Journal of Earth and Environmental Sciences* 8:43–48.
- Goldyn, R., S. Podsiadłowski, R. Dondajewska, and A. Kozak. 2014. The sustainable restoration of lakes – towards the challenges of the Water Framework Directive. *Ecohydrology & Hydrobiology* 14:68–74.
- Hall, K. J., T. P. D. Murphy, M. Mawhinney, and K. I. Ashley. 1994. Iron treatment for eutrophication control in Black Lake, British Columbia. *Lake and Reservoir Management* 9:114–117.
- Hayes, C. R., R. G. Clark, R. F. Stent, and C. J. Redshaw. 1984. The control of algae by chemical treatment in a eutrophic water supply reservoir. *Journal of the Institution of Water and Environment Management* 38:149–162.
- Hudnell, H. K. 2010. The state of U.S. freshwater harmful algal blooms assessments, policy and legislation. *Toxicon* 55:1024–1034.
- Jaeger, D. 1994. Effects of hypolimnetic water aeration and iron-phosphate precipitation on the trophic level of Lake Krupunder. *Hydrobiologia* 275/276:433–444.
- Jiang, Y., B. Ji, R. N. S. Wong, and M. H. Wong. 2008. Statistical study on the effects of environmental factors on the growth and microcystins production of bloom-forming cyanobacterium – *Microcystis aeruginosa*. *Harmful Algae* 7:127–136.
- Katsev, S., I. Tsandev, I. L'Heureux, and D. G. Rancourt. 2006. Factors controlling long-term phosphorus efflux from lake sediments: exploratory reactive-transport modeling. *Chemical Geology* 234:127–147.
- Kleeberg, A., A. Köhler, and M. Hupfer. 2012. How effectively does a single or continuous iron supply affect the phosphorus budget of aerated lakes? *Journal of Soils and Sediments* 12:1593–1603.
- Kleeberg, A., C. Herzog, and M. Hupfer. 2013. Redox sensitivity of iron in phosphorus binding does not impede lake restoration. *Water Research* 47:1491–1502.
- Klein, A. R., D. S. Baldwin, and E. Silvester. 2013. Proton and iron binding by the cyanobacterial toxin microcystin-LR. *Environmental Science & Technology* 47:5178–5184.
- Kowalczywska-Madura, K., R. Dondajewska, and R. Goldyn. 2008. Influence of iron treatment on phosphorus internal loading from bottom sediments of the restored lake. *Limnological Review* 8:177–182.
- Kozak, A., and R. Goldyn. 2014. Variation in phyto- and zooplankton of restored Lake Uzarzewskie. *Polish Journal of Environmental Studies* 23:1201–1209.

- Kranzler, C., M. Rudolf, N. Keren, and E. Schleif. 2013. Iron in cyanobacteria. Pages 57–105 in F. Chauvat and C. Cassier-Chauvat, editors. *Advances in botanical research: genomics of cyanobacteria*. Volume 65. Academic Press, Amsterdam, Netherlands.
- Li, X., D. Elliott, and W. Zhang. 2006. Zero-valent iron nanoparticles for abatement of environmental pollutants: materials and engineering aspects. *Critical Reviews in Solid State and Material Sciences* 31:111–122.
- Li, H., T. Murphy, J. Guo, T. Parr, and C. Nalewajko. 2009. Iron-stimulated growth and microcystin production of *Microcystis novacekii* UAM 250. *Limnologia* 39:255–259.
- Lukac, M., and R. Aegerter. 1993. Influence of trace metals on growth and toxin production of *Microcystis aeruginosa*. *Toxicon* 31:293–305.
- Manning, P. G., T. P. Murphy, and E. E. Prepas. 1991. Intensive formation of vivianite in the bottom sediments of mesotrophic Narrow Lake, Alberta. *Canadian Mineralogist* 29:77–85.
- Maranger, R., C. D. Canham, M. L. Pace, and M. J. Papaik. 2006. A spatially explicit model of iron loading to lakes. *Limnology and Oceanography* 51:247–256.
- Marsalek, B., D. Jancula, E. Marsalkova, M. Mashlan, K. Safarova, J. Tucek, and R. Zboril. 2012. Multimodal action and selective toxicity of zerovalent iron nanoparticles against cyanobacteria. *Environmental Science & Technology* 46:2316–2323.
- Martin, J. H., et al. 1994. Testing the iron hypothesis in ecosystems of the equatorial Pacific Ocean. *Nature* 371:123–129.
- Michard, G., G. Sarazin, D. Jézéquel, P. Albéric, and S. Ogier. 2001. Annual budget of chemical elements in a eutrophic lake, Aydat lake (Puy-de-Dôme), France. *Hydrobiologia* 459:27–46.
- Molot, L. A., G. Li, D. L. Findlay, and S. B. Watson. 2010. Iron-mediated suppression of bloom-forming cyanobacteria by oxine in a eutrophic lake. *Freshwater Biology* 55:1102–1117.
- Molot, L. A., S. B. Watson, I. F. Creed, C. G. Trick, S. K. McCabe, M. J. Verschoor, R. J. Sorichetti, C. Powe, J. J. Venkiteswaran, and S. L. Schiff. 2014. A novel model for cyanobacteria bloom formation: the critical role of anoxia and ferrous iron. *Freshwater Biology* 59:1323–1340.
- Murphy, T., A. Lawson, M. Kumagai, and C. Nalewajko. 2001. Release of phosphorus from sediments in Lake Biwa. *Limnology* 2:119–128.
- Nembrini, G. P., J. A. Capobianco, M. Viel, and A. F. Williams. 1983. A Mössbauer and chemical study of the formation of vivianite in sediments of Lago Maggiore (Italy). *Geochimica et Cosmochimica Acta* 47:1459–1464.
- Nriagu, J. O. 1972. Stability of vivianite and ion-pair formation in system $\text{Fe}_3(\text{PO}_4)_2$ – $2\text{H}_3\text{PO}_4$ – H_2O . *Geochimica et Cosmochimica Acta* 36:459–470.
- Nürnberg, G. K., and P. J. Dillon. 1993. Iron budgets in temperate lakes. *Canadian Journal of Fisheries and Aquatic Sciences* 50:1728–1737.
- Orihel, D. M., D. W. Schindler, N. C. Ballard, M. D. Graham, D. W. O'Connell, L. R. Wilson, and R. D. Vinebrooke. 2015. The “nutrient pump”: Iron-poor sediments fuel low nitrogen-to-phosphorus ratios and cyanobacterial blooms in polymictic lakes. *Limnology and Oceanography* 60:856–871.
- Peelen, R. 1969. Possibilities to prevent blue-green algal growth in the Delta region of the Netherlands. *Verhandlungen Internationale Vereinigung für Theoretische und Angewandte Limnologie* 17:763–766.
- Pollinger, U., B. Kaplan, and T. Berman. 1995. The impact of iron and chelators on Lake Kinneret phytoplankton. *Journal of Plankton Research* 17:1977–1992.
- Quaak, M., J. van der Does, P. Boers, and J. van der Vlugt. 1993. A new technique to reduce internal phosphorus loading by in-lake phosphate fixation in shallow lakes. *Hydrobiologia* 253:337–344.
- Rappold, T. A., and K. S. Lackner. 2010. Large scale disposal of waste sulfur: from sulfide fuels to sulfate sequestration. *Energy* 35:1368–1380.
- Riley, E. T., and E. E. Prepas. 1984. Role of internal phosphorus loading in two shallow, productive lakes in Alberta, Canada. *Canadian Journal of Fisheries and Aquatic Sciences* 41:845–855.
- Ripl, W. 1976. Biochemical oxidation of polluted lake sediment with nitrate – a new lake restoration method. *Ambio* 5:132–135.
- Ripl, W. 1978. Oxidation of lake sediments with nitrate – a restoration method for former recipients. Pages 1–151. Institute of Limnology, University of Lund, Lund, Sweden.
- Roden, E. E., and J. W. Edmonds. 1997. Phosphate mobilization in iron-rich anaerobic sediments: microbial Fe(III) oxide reduction versus iron-sulfide formation. *Archiv für Hydrobiologie* 139:347–378.
- Rothe, M., T. Frederichs, M. Eder, A. Kleeberg, and M. Hupfer. 2014. Evidence for vivianite formation and its contribution to long-term phosphorus retention in a recent lake sediment: a novel analytical approach. *Biogeosciences* 11:5169–5180.
- Rozan, T. F., M. Taillefert, R. E. Trouwborst, B. T. Glazer, S. Ma, J. Herszage, L. M. Valdes, K. S. Price, and G. W. III Luther. 2002. Iron-sulfur-phosphorus cycling in the sediments of a shallow coastal bay: implications for sediment nutrient release and benthic macroalgal blooms. *Limnology and Oceanography* 47:1346–1354.
- Schindler, D. W. 1977. Evolution of phosphorus limitation in lakes. *Science* 195:260–262.
- Schindler, D. W. 2012. The dilemma of controlling cultural eutrophication of lakes. *Proceedings of the Royal Society B* 279:4322–4333.
- Schindler, D. W., and W. F. Donahue. 2006. An impending water crisis in Canada's western prairie provinces. *Proceedings of the National Academy of Sciences of the United States of America* 103:7210–7216.
- Schindler, D. W., R. E. Hecky, D. L. Findlay, M. P. Stainton, B. R. Parker, M. J. Paterson, K. G. Beaty, M. Lyng, and S. E. M. Kasian. 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.
- Sevilla, E., B. Martin-Luna, L. Vela, M. T. Bes, M. F. Fillat, and M. L. Peleato. 2008. Iron availability affects *mcvD* expression and microcystin-LR synthesis in *Microcystis aeruginosa* PCC7806. *Environmental Microbiology* 10:2476–2483.
- Shaked, Y., Y. Erel, and A. Sukenik. 2004. The biogeochemical cycle of iron and associated elements in Lake Kinneret. *Geochimica et Cosmochimica Acta* 68:1439–1451.
- Smith, V. H., and S. J. Bennett. 1999. Nitrogen: phosphorus supply ratios and phytoplankton community structure in lakes. *Archiv für Hydrobiologie* 146:37–53.
- Smith, V. H., and D. W. Schindler. 2009. Eutrophication science: where do we go from here? *Trends in Ecology and Evolution* 24:201–207.
- Smolders, A., and J. G. M. Roelofs. 1993. Sulfate-mediated iron limitation and eutrophication in aquatic ecosystems. *Aquatic Botany* 46:247–253.

- Smolders, A. J. P., L. P. M. Lamers, E. C. H. E. T. Lucassen, G. Van Der Velde, and J. G. M. Roelofs. 2006. Internal eutrophication: how it works and what to do about it – a review. *Chemistry and Ecology* 22:93–111.
- Sobczyński, T., T. Joniak, and E. Pronin. 2012. Assessment of the multi-directional experiment to restore Lake Góreckie (western Poland) with particular focus on oxygen and light conditions: first results. *Polish Journal of Environmental Studies* 21:1025–1031.
- Sohm, J. A., E. A. Webb, and D. G. Capone. 2011. Emerging patterns of marine nitrogen fixation. *Nature Reviews – Microbiology* 9:499–508.
- Søndergaard, M., R. Bjerring, and E. Jeppesen. 2013. Persistent internal phosphorus loading during summer in shallow eutrophic lakes. *Hydrobiologia* 710:95–107.
- Stern, D. I. 2005. Global sulfur emissions from 1850 to 2000. *Chemosphere* 58:163–175.
- Takenaka, S., and Y. Tanaka. 1995. Decomposition of cyanobacterial microcystins by iron(III) chloride. *Chemosphere* 30:1–8.
- Taranu, Z. E., et al. 2015. Acceleration of cyanobacterial dominance in north temperate-subarctic lakes during the Anthropocene. *Ecology Letters* 18:375–384.
- Till, A. R. 2010. Sulphur and sustainable agriculture. International Fertilizer Industry Association, Paris, France.
- Twiss, M. R., J. C. Auclair, and M. N. Charlton. 2000. An investigation into iron-stimulated phytoplankton productivity in epipelagic Lake Erie during thermal stratification using trace metal clean techniques. *Canadian Journal of Fisheries and Aquatic Sciences* 57:86–95.
- Utkilen, H., and N. Gjølme. 1995. Iron-stimulated toxin production in *Microcystis aeruginosa*. *Applied and Environmental Microbiology* 61:797–800.
- Van der Welle, M. E. W., A. J. P. Smolders, H. J. M. Op den Camp, J. G. M. Roelofs, and L. P. M. Lamers. 2007. Biogeochemical interactions between iron and sulphate in freshwater wetlands and their implications for interspecific competition between aquatic macrophytes. *Freshwater Biology* 52:434–447.
- Van der Zee, C., D. R. Roberts, D. G. Rancourt, and C. P. Slomp. 2003. Nanogoethite is the dominant reactive oxyhydroxide phase in lake and marine sediments. *Geology* 31:993–996.
- Vuorio, K., A. Lagus, J. M. Lehtimäki, J. Suomela, and H. Helminen. 2005. Phytoplankton community responses to nutrient and iron enrichment under different nitrogen to phosphorus ratios in the northern Baltic Sea. *Journal of Experimental Marine Biology and Ecology* 322:39–52.
- Walker, W. W. Jr, C. E. Westerberg, D. J. Schuler, and J. A. Bode. 1989. Design and evaluation of eutrophication control measures for the St. Paul water supply. *Lake and Reservoir Management* 5:71–83.
- Wolter, K.-D. 2010. Restoration of eutrophic lakes by phosphorus precipitation, with a case study on Lake Gross-Glienicker. Pages 85–99 in M. Eiseltová, editor. *Restoration of lakes, streams, floodplains, and bogs in Europe: principles and case studies*. Springer, Dordrecht, Netherlands.
- Wurtsbaugh, W. A., and A. J. Horne. 1983. Iron in eutrophic Clear Lake, California: its importance for algal nitrogen fixation and growth. *Canadian Journal of Fisheries and Aquatic Sciences* 30:1419–1429.
- Xu, H., G. Zhu, B. Qin, and H. W. Paerl. 2012. Growth response of *Microcystis* spp. to iron enrichment in different regions of Lake Taihu, China. *Hydrobiologia* 700:187–202.
- Young, S. N., W. T. Clough, A. J. Thomas, and R. Siddall. 1988. Changes in plant community at Foxcote Reservoir following use of ferric sulfate to control nutrient levels. *Water and Environment Journal* 2:5–12.
- Zhu, M.-X., J. Liu, G.-P. Yang, T. Li, and R.-J. Yang. 2012. Reactive iron and its buffering capacity towards dissolved sulfide in sediments of Jiaozhou Bay, China. *Marine Environmental Research* 80:46–55.

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