Supplementary Information for

Sustained stoichiometric imbalance and its ecological consequences in a large oligotrophic lake

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Supplemental Information

Nitrate dynamics in summer

To demonstrate the summertime depletion of nitrate (NO$_3^-$) in the surface waters of Flathead Lake, we plotted concentrations of NO$_3^-$ at 5 m depth in 2018 and 2019, the years when the phytoplankton bioassays were performed. The data show that by the end of July, NO$_3^-$ levels are reduced to levels below the limit of detection (1.5 ug L$^{-1}$ NO$_3^-$N, dotted line). The grey area highlights the period during which the N and P enrichment experiments were performed.

![Nitrate concentrations in Flathead Lake](image)

**Figure S1.** Nitrate + nitrite concentrations in the surface waters (5 m) of Flathead Lake during summer 2019 and summer 2020. The dates of the six bioassay experiments are indicated with vertical arrows.

Silica correction of total P data

The presence of soluble silicon, presented here as silica (SiO$_2$) in natural waters produces a positive interference with P colorimetry (1,2). The implementation of new laboratory instrumentation and therefore reaction kinetics at the FLBS analytical chemistry laboratory in 2013 required a revalidation of this potential interference. Given the high concentration of silica (~5 mg SiO$_2$ L$^{-1}$) in relation to the PO$_4^{3-}$-depleted waters of Flathead Lake, a lower pH color reagent was introduced to the methodology to successfully eliminate any potential silica interference on P quantification (3). Due to the fact that sample pH approaches 2 or lower during most TP digestion procedures, this change wasn't expected to produce a change in TP quantification.

However, when examining the long-term P data for this analysis we observed a consistent, albeit modest, decline in TP concentration as well as decreased temporal variability that coincided with new analytical procedures being implemented in 2013. Given that the known silica concentration of Flathead Lake has been stable at 5 mg SiO$_2$ L$^{-1}$ historically, we posited that if we could quantify the interference we could make a correction to the historical data to obtain an accurate assessment of TP dynamics for the entire sampling period.
To quantify the historical silica interference, we conducted an experiment to vary the TP and silica concentrations across a large range of expected values. The experiment was conducted by adding SiO$_2$ (as sodium metasilicate) to deionized water and digesting the samples using an alkaline persulfate wet chemical oxidation (4). Digested samples were then analyzed colorimetrically (5) using both the traditional reagent chemistry used in the laboratory and then a reagent chemistry with a pH that specifically suppresses the silica interference. Samples analyzed without silica suppression chemistry showed both increased “apparent” P with increasing silica as well as much higher variability (Figure S2).
Figure S2. Measured total phosphorus increased with added SiO$_2$ in uncorrected (red points) samples. Silica had no clear effect on TP when corrected for silica interference. Note also the increase in variability for the uncorrected samples.
We then assessed the cause of the high variability in the silica-uncorrected samples by directly quantifying the SiO$_2$ concentrations of the digested samples (6). We found that relatively high concentrations of silica (up to 30 mg SiO$_2$ L$^{-1}$) were leaching from the glass vials used for digestion. Using the uncorrected samples, we regressed the TP anomaly (i.e., measured TP minus the TP spike added) against the measured silica concentration (this concentration included both the SiO$_2$ spike and the amount that leached from the vials).

The measured TP anomaly was strongly positively related with measured silica (Figure S3), showing strong interference from silica. We fit a linear model to these data assuming Student’s t-distributed errors (i.e. robust regression that allows less impact from outliers). This model was TP = -11.25 + 0.33[SiO$_2$] + ε, showing that each mg L$^{-1}$ of SiO$_2$ increased TP by 0.33. Thus, given an average (and relatively invariant) concentration of Si of 4.8 mg SiO$_2$ L$^{-1}$ in the lake implies a correction of 1.6 µg P L$^{-1}$ for lake TP samples. This correction was applied to all lake water samples that were analyzed prior to mitigation of silica interference. Silica concentrations in river samples were higher and more variable than in the lake. Thus, we corrected each measurement of river P concentration during the period prior to mitigation of silica interference using the equation TP = -11.25 + 0.33[SiO$_2$] + ε. Figures 1 and 2 in the main text depict the silica-corrected values.
Figure S2. Magnitude of the observed anomaly in P concentration (the difference between P concentration measured and known P concentration) and the measured concentration of silica in solution in the reaction vessel.
SI References

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