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From pipes to streams: The hidden influence of orthophosphate additions on urban waterways

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Abstract

We present clear evidence that orthophosphate (PO_4^{3-}), commonly used by drinking water treatment plant (DWTP) operators to reduce lead pipe corrosion, can move beyond its intended domain and enter urban stream networks. This migration influences stream biogeochemistry in ways previously undocumented. In collaboration with a DWTP in Pittsburgh, Pennsylvania, we conducted a study across five urban streams, capturing pre- and post-implementation phases of PO_4^{3-} -based corrosion control. Through comprehensive chemical analyses of nutrients, anions, metals, and nitrate isotopes, as well as nutrient limitation bioassays with *Cylindrospermopsis* sp. and *Raphidocelis subcapitata*, we demonstrate statistically robust increases in streamwater phosphorous concentrations (total dissolved phosphorus: $p < 0.0001$; total reactive phosphorus and total phosphorus: $p < 0.05$). These shifts coincide with elevated dissolved metal concentrations, implicating corrosion control byproducts as co-transported constituents. Principal Component Analysis reveals urban stream chemistry is governed by a complex interplay of solutes derived from PO_4^{3-} -treated drinking water, pipe corrosion, mineral weathering, and wastewater. Bioassays confirm that nutrient additions, particularly P and NP, significantly stimulate algal biomass ($p < 0.05$) with *Cylindrospermopsis* sp. showing heightened responsiveness to N additions shortly after PO_4^{3-} deployment. These findings expose a critical, underrecognized pathway by which drinking water infrastructure contributes to nutrient enrichment and eutrophication in urban aquatic systems. The implications are clear: subsurface infrastructure is not a closed system, and its chemical footprint extends into the urban hydroscape with ecological consequences that warrant closer attention.

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Introduction

Recent public health emergencies in urban areas of the U.S. stemmed from corrosion of plumbing materials containing lead (Pb), which released both particulate and dissolved forms of Pb into drinking water [1]. The contamination of drinking water with Pb and the related health concerns have attracted significant attention following the recent health crises in cities such as Flint, Michigan, Washington D.C, and Pittsburgh, Pennsylvania (e.g., [2–6]). Beyond documented case studies in these cities, an estimated 15–22 million people are served by community water systems in the U.S. that contain full or partial Pb service lines, with the highest prevalence in the midwestern U.S. and Great Lakes states [7]. The U.S. EPA's Lead and Copper Rule states that the maximum contaminant level goal is 0 μg/L, emphasizing that there is no known safe blood Pb level, whereas the action level is 10 μg/L for Pb in drinking water [8].

Given the sustained challenges of controlling Pb solubility in drinking water pipe networks, many community water systems in North America [9] and parts of Europe, including the UK [10], use phosphate corrosion inhibitors, including blended polyphosphates, orthophosphate (abbreviated as PO_4^{3-} but also referred to as Soluble Reactive Phosphorus or SRP), or zinc orthophosphate [11]. PO_4^{3-} treatment induces the formation of a relatively insoluble lead-bearing scale on the interior pipe walls that reduces Pb release [1,11,12]. Lead pipes naturally develop a passivating layer (i.e., protective layer on a metal surface that is resistant to corrosion) often composed of lead carbonate or lead oxide [13]. When PO_4^{3-} is introduced, it interacts with this layer, potentially dissolving or weakening it before a new protective layer forms. While the public health benefits of dosing drinking water systems with scale-forming PO_4^{3-} cannot be overstated, the potential ecological consequences of PO_4^{3-} dosing of drinking water systems to streams, rivers, and groundwater are largely unexplored, particularly in the U.S. [14].

The transfer of phosphorus (P) from land to aquatic environments, exacerbated by human activities, has raised concern among researchers and policymakers because of its detrimental effects on water quality [15]. Phosphorus is a limiting nutrient in some aquatic ecosystems, and PO_4^{3-} is the most biologically available form of P in lakes, streams, and rivers [16–19]. Ecological studies have found that P concentrations in the water column can control primary producer assemblage in streams [20]. Moreover, there is conclusive evidence that anthropogenic P and nitrogen (N) enrichment are linked with changes in phytoplankton biomass [20,21], including green algae and toxic cyanobacteria in streams [22]. Anthropogenic inputs of P, either from point sources or diffuse sources, can have substantial effects on urban surface water and ultimately lead to a range of social, economic, and environmental problems [23]. However, the development of effective strategies for reducing total phosphorus (TP) impacts to aquatic ecosystems is challenged by a limited understanding of spatial and temporal variations in P fluxes from non-point sources in watersheds [24]. To address these challenges, it is crucial to deepen our understanding of P movement across landscapes and within ecosystems [25].

Although P dynamics originating from fertilizer inputs have been extensively documented in agricultural areas, the second and third largest sources of P exported

to waterways in the continental U.S. are sewage and point sources, respectively, and both are concentrated in urban areas [25]. In addition to point source inputs from industrial discharges and wastewater treatment plant effluent, non-point sources of P in urban areas include atmospheric deposition, lawn fertilizers, pet waste, and leaking sewers [24]. In particular, leaking sewer infrastructure, designed to convey domestic loads of P from human waste, food additives, and food waste as greywater or blackwater, could be significant non-point P sources although their relative magnitudes are unknown [25].

Unintended leakage of PO_4^{3-} -dosed drinking water from aging or compromised water mains is among the least understood contributors to urban P flux [26]. Water utilities report variable rates of lost or "unaccounted" for treated drinking water [27,28]. For example, 25–27% of treated drinking water is estimated to leak from supply systems in England and Wales [29], where unintentional P loadings from pipe networks were associated with pipe density [30]. In the U.S., P dosing added 14.9 kt PO_4-P yr^{-1} to the water distribution network in 2015, however 17% and 21% of this total load was lost due to water mains leakage and outdoor water use, respectively [26]. In fact, P fluxes from combined leakage and outdoor water use have exceeded point source P inputs to freshwater across several counties in the U.S. [26]. Accounting for this poorly constrained P source is increasingly important as water infrastructure systems continue to age. For example, water main breakage rates increased by 27% from 2012 to 2018 in the U.S. and Canada and breakage rates are projected to continue increasing [31]. In the U.S., an estimated PO_4^{3-} flux of 0.7 to 2.6 ktPO_4-P per year is lost due to water mains leakage [26]. Despite the magnitude of this potential surplus source of P loading to U.S. surface water from drinking water networks, field scale impacts to urban surface water and nutrient limitation remain generally unexplored, particularly in the U.S.

Water dosed with PO_4^{3-} at a drinking water treatment plant can impact urban aquatic systems through multiple pathways (Fig 1). First, pressurized, underground drinking water pipe networks in cities are subject to degradation, breakage, and deferred maintenance as they age. Exfiltration of P-dosed drinking water from pipes to the subsurface can infiltrate groundwater and increase baseflow P fluxes to streams or reach streams through macropore flow pathways, subsurface drainage, or aquifer discharge. Secondly, PO_4^{3-} dosed drinking water can enter stream networks through sewer system leaks. When treated drinking water enters homes and businesses, it is used for drinking, washing, flushing waste, and other daily activities. After use, drinking water is combined with raw sewage and enters gravity-fed sewer systems where stream-sewer connections are common and sometimes, streams are buried inside of sewer networks [32]. Deferred maintenance of sewer infrastructure, coupled with the close proximity of sewer networks to streams, can increase pathogen and nutrient loads to streams [33] as documented with N mass balance studies [33] and surveys of nitrate (NO_3^-) isotopes in streams during baseflow [34] and stormflow [35]. However, despite the documented occurrence of sewage-derived NO_3^- in some urban streams (e.g., [35,36]), the extent to which these leaky conditions impact P concentrations and nutrient limitation in urban streams remains poorly constrained. Thirdly, sewer networks convey raw sewage in a slurry of treated drinking water as influent to wastewater treatment plants where treatment procedures can vary. Treated wastewater is clarified, aerated, and disinfected before effluent is discharged to surface water, where remaining P and N in effluent can exacerbate nutrient loads to urban rivers (Fig 1).

In this study, we collaborated with the Pittsburgh Water and Sewer Authority (PWSA) (Pittsburgh, PA, USA) to evaluate the potential ecological impact to urban streams following the initiation of PO_4^{3-} dosing. The PWSA began introducing orthophosphoric acid into approximately 70 million gallons of drinking water through a phased approach starting on April 1, 2019 where the median dosage concentration was 630 µg P/L. This dosing concentration is over 50-times higher than the lower 25th percentile TP concentrations (12 µg P/L) observed in over 7,000 Pennsylvania streams [37]. This dosing concentration is also an order of magnitude greater than a P impairment threshold of 50 µg P/L documented for headwater streams in Great Britain [17]. Concurrently, the Pittsburgh drinking water pipe network loses a high rate of treated drinking water due to leaks in aging infrastructure, unmetered use, and water main breaks (approximately 40–50% of the 70 million gallons per day) [38].

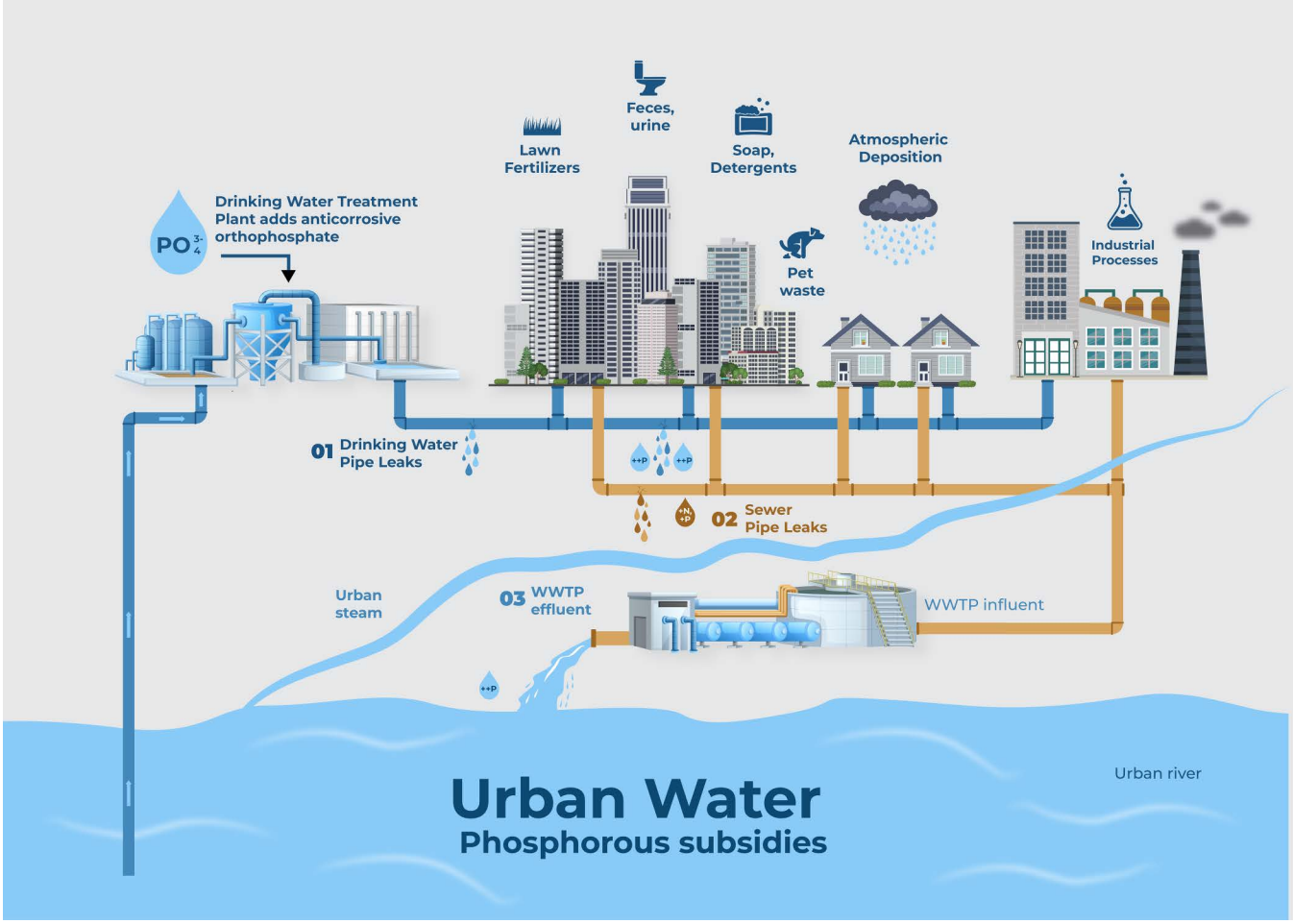


Fig 1. Conceptual model of three major points of interaction amongst the drinking water (DW) network, built, and natural water systems. To prevent corrosion of lead from drinking water pipes, the Drinking Water Treatment Plant (DWTP) operators add phosphate as H_3PO_4 to the pressurized pipe network where the P-enriched DW is distributed. 1) The aging pipe network results in high leakage rates and exfiltration of P-enriched DW to the urban subsurface, where an unknown amount of P-enriched DW leaches to urban streams. 2) After P-enriched DW is used in homes for drinking, showering, laundry, etc., it enters the sewer pipe network as wastewater (WW). Breaks, failures, and cracks in the sewer network cause exfiltration of an unknown amount of WW to the urban subsurface, where an unknown amount of P-enriched WW leaches to urban streams. 3) WW is conveyed as influent to the Wastewater Treatment Plant (WWTP). After WW treatment, the WWTP discharges effluent to urban rivers where residual P contributes to nutrient impairment.

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Here, we explore whether leaks in the drinking water distribution network are an additional source of P in an urban stream network that was largely buried in the stormwater pipe network during urbanization [33]. Prior work in the City of Pittsburgh, Pennsylvania, has documented the dominance of leaking sewers across flow conditions as the dominant NO_3^- source to a restored urban stream, Nine Mile Run, where much previous research has taken place [34–36]. However, beyond Nine Mile Run, it remains unexplored the extent to which subsurface built infrastructure, both drinking and sewer networks, impact other urban streams throughout the Pittsburgh region. To investigate the potential hydrologic connectivity between the drinking water pipe networks with urban streams, we document changes in monthly stream chemistry in a suite of five urban stream reaches in the City of Pittsburgh, Pennsylvania that span pre-treatment conditions (March 2019), treatment initiation (April 2019), and post-treatment conditions (May 2019

- June 2020). Solutes measured include concentrations of nutrients, dissolved metals, anions, and nitrate isotopes, and are compared to the solute chemistry of samples extracted from the drinking water distribution network. Additionally, to investigate whether drinking water PO_4^{3-} additions impact stream ecology and to assess potential ecosystem responses, we performed nutrient addition bioassays using *Cylindrospermopsis* sp. and *Raphidocelis subcapitata* using surface water from the same five urban streams before and after PO_4^{3-} based corrosion controls were added to drinking water. These nutrient manipulation bioassays are designed to document whether phytoplankton responds, and if so, how quickly, to changing thresholds of nutrient concentrations in tap and streamwater. Lastly, we evaluate the broader impact to river ecosystems by examining changes in P flux to and from the WWTP receiving sewage influent containing PO_4^{3-} dosed drinking water. Uncovering this information is an important step in understanding controls on urban stream and riverine biogeochemistry and dynamics, that are also highly impacted by nonpoint source contributions of wastewater nitrogen inputs [39].

Methods

Study location

Like many other cities, streams in Pittsburgh were buried in the stormwater pipe network in the early 1900s [32–33]. For this study, we chose five remaining above-ground urban streams for study sites (Fig 2). Water samples were collected from above-ground reaches of each stream in the PWSA drinking water service area: Fern Hollow (Fern), Negley Run (Negley), Panther Hollow (Panther), Phipps Run (Phipps), and Shades Run (Shades), characterized by different land uses and population densities (S1 Table). Negley Run watershed is the most intensively developed and has the highest population density. In contrast, the Phipps Run watershed has the most open space due to the presence of a golf course. Panther Hollow and Fern Hollow watersheds are moderately developed, while Shades Run watershed is the most forested among the five watersheds (S1 Table).

P-dosing, pipe network and stream sampling

The PWSA began introducing orthophosphoric acid into approximately 70 million gallons of drinking water at seven locations within the distribution network through a phased approach starting on April 1, 2019. All drinking water customers received PO_4^{3-} treated water to reduce lead corrosion in water service lines [40] by the end of April 2019. As a result, all drinking water distribution system locations in our study received treated drinking water by April 24, 2019 (Fig 2). To achieve a targeted tap water PO_4^{3-} concentration of 158 µg P/L and promote sufficient scale formation within the PWSA pipe network, PO_4^{3-} was initially dosed at 1040 µg P/L in April 2019. The dosage was subsequently reduced to a final concentration of 630 µg P/L by September 2019, with dosing continuing to the present [41]. On-site PO_4^{3-} concentrations were measured in samples collected monthly from five locations throughout the drinking water distribution network over the study period (from February 2019 to February 2020) by PWSA using a portable DR900 spectrophotometer with a method detection limit of 3 µg P/L (Hach, Loveland, CO, USA) (Fig 2).

We identified five non-buried urban stream reaches for sample collection. Stream reaches were 1st or 2nd order shallow streams generally 6–12 inches deep (S1 Table). Surface water sampling was conducted at least monthly from February 2019 to June 2020 to assess streamwater chemistry before and after PO_4^{3-} addition to the drinking water distribution system. To characterize stream chemistry, one liter of streamwater was collected from streams using acid-washed and sample-rinsed polypropylene bottles. Surface water samples were collected mid-stream away from the stream bank and at mid-depth, facing upstream, using a one-liter bottle attached to a pole to avoid stirring the sediment-water interface. To ensure that samples represented dry weather conditions, at least three days of dry weather was required prior to sample collection to capture baseflow rather than precipitation-influenced stormflows.

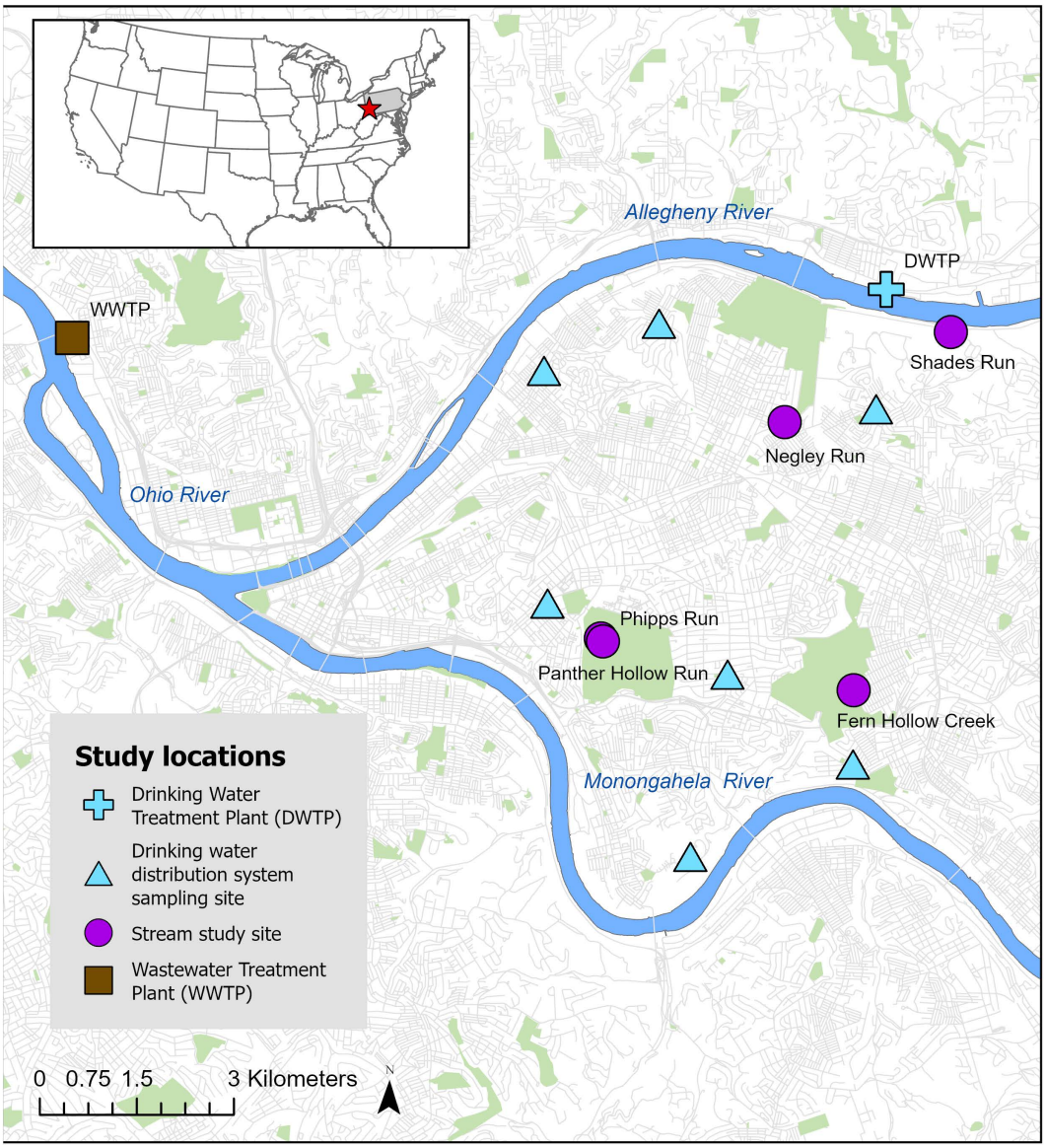


Fig 2. Urban stream sampling locations, drinking water distribution system sampling sites, the Drinking Water Treatment Plant (DWTP), and the Wastewater Treatment Plant (WWTP) in the City of Pittsburgh, Pennsylvania (U.S.).

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Chemical characterization

Phosphorus exists in numerous chemical forms within natural water systems [42,43], where the distinction between 'dissolved' and 'particulate' P phase is based on 0.45 μm filtration [44]. Here, we characterize four major forms of P, including two dissolved or "soluble" species and two "total" species comprised of both dissolved and particulate P. Soluble Reactive Phosphorus (SRP) measures inorganic P in solution, also known as orthophosphate (PO_4^{3-}). It is readily assimilated by phytoplankton and generally serves as a proxy for bioavailable P. SRP is also the dominant form of P found in wastewater [45]. Total Reactive Phosphorus (TRP) measurements are made on unfiltered and undigested samples, and refer to bioavailable P, including SRP as well as reactive particulate P. Total Dissolved Phosphorus (TDP), also referred to as Total

Soluble Phosphorus contains both SRP and hydrolyzable, organic forms of P [44]. Total Phosphorus (TP) refers to the sum of TDP plus particulate P and is measured on an unfiltered sample after digestion that converts recalcitrant or bound P to a measurable inorganic form. TP includes both bioavailable (SRP, TRP) and unavailable (organic P and particulate P) forms.

Stream samples were analyzed for pH and concentrations of nutrients, anions, dissolved metals, and nitrate isotopes. Samples were filtered within 24h of collection using 0.45 μm polyethersulfone membrane syringe filters (Thermo Fisher Scientific, Waltham, MA) for the measurement of nitrate and nitrite and PO_4^{3-} concentrations [43]. Because nitrite concentrations were below the detection limit (1 μg N/L) for all samples, measurements of nitrite + nitrate are hereafter referred to as nitrate (NO_3^-). For characterization of dissolved metals, as well as TDP, sample splits were acidified using sub-boil distilled, concentrated nitric acid and stored at –20°C. These samples were diluted with 2% nitric acid and spiked with an internal standard. Diluted and spiked samples were analyzed on a PerkinElmer Nexion 300X Inductively Coupled Plasma-Mass Spectrometer (ICP-MS). Measurements for NO_3^-, total nitrogen (TN), TRP, SRP, and TP were conducted on a Lachat QuickChem Analyzer. The Lachat was calibrated using standard solutions of the analyte of interest prior to each run. Check standards and blanks were used when unusual peak shapes, baseline drift, and noise were detected. Additionally, sample duplicates were used to estimate precision. Silica was found to interfere with PO_4^{3-} measurements in our streamwater samples, so a modified version of the QuickChem method 10-115-01-1-Y, which is equivalent to Environmental Protection Agency 365.1, was used. This method reduced the heater temperature from 60°C to 37°C and increased the proportion of concentrated sulfuric acid used in the molybdenum color reagent from 72 ml/L to 95 ml/L [46]. Fluoride (F^-), chloride (Cl^-), and sulfate (SO_4^{2-}) ion concentrations were measured on a Dionex ICS2000 Ion Chromatograph. Dual nitrogen and oxygen isotopes of dissolved nitrate ($\delta^{15}N\text{-}NO_3^-$, $\delta^{18}O\text{-}NO_3^-$) were measured on an IsoPrime Continuous Flow Mass Spectrometer following the denitrifier method at the Pitt Isotope Tracers Lab at the University of Pittsburgh [47–50]. We report ratios of TN:TP as molar ratios.

Due to sampling constraints and changes in project personnel, sampling periods for some constituents were slightly different. TRP, TP, and TN were measured at least monthly from 2/2019–6/2020, whereas TDP, dissolved metals, and anions were measured at least monthly from 3/2019–6/2020. SRP and NO_3^- isotopes were measured monthly from 7/2019–6/2020, whereas NO_3^- was measured monthly from 6/2019–6/2020. Given that TP, TRP and SRP were measured using colorimetric methods, while TDP values were determined through ICP-MS analysis, the potential differences in these analytical techniques could impact the comparability of results and thus the sum of P species can exceed TP concentrations [51].

Nutrient addition bioassay

Surface water samples were collected from the five urban stream sampling locations for identical nutrient addition bioassays at three time points. The first bioassay was conducted in March 2019 (before PO_4^{3-} addition), while the two other bioassays were conducted in June 2019 and April 2020 (two and 12 months after PO_4^{3-} addition, respectively). Four liters of streamwater were collected from each sampling location into acid-washed and sample-rinsed polyethylene bottles for each algal assay. We used a control, referred to as “streamwater control” that received no nutrient amendments.

Concurrently, four liters of PO_4^{3-} dosed tap water were collected from our laboratory (University of Pittsburgh). In the tap water bioassays, tap water, free from naturally-occurring algae, including both cyanobacteria and green algae, and N, is used to understand the impact of variable tap water PO_4^{3-} concentrations on phytoplankton growth. The tap water control from March 2019 contained no drinking water PO_4^{3-}. In contrast, the “tap water control” at 2- and 12- months was enriched with drinking water PO_4^{3-} for the assays but had no additional nutrient amendments.

In all bioassay experiments, three treatments, in addition to the control, were administered for each assay: N addition (+N), P addition (+P), and N and P addition (+NP). N was added as KNO_3, while P was added as $K_2HPO_4 \cdot 3H_2O$ [52]. During the study period, ammonium (NH_4^+) concentrations were below detection (10 μg N/ L) for all samples and in all

sampling locations. Thus, we added N as NO_3^- instead of NH_4^+, although phytoplankton preferentially takes up NH_4^+ due to the energetic cost of reducing NO_3^- to NH_4^+ [53]. The + N, + P, and +NP treatments were amended each with a range of concentrations: + N only: 500, 1000, 2000, 5000 µg N/L; + P only: 50, 100, 200, 500 µg P/L; + PN: 50P plus 500 N; 100 P plus 1000N; 200 P plus 2000N; 500P plus 5000N µg/L, respectively. Our highest P amendment (500 µg P/L) was similar to the final dosing concentration used by PWSA (630 µg P/L).

Cultured green algae (*Raphidocelis subcapitata,* formerly *Selenastrum capricornutum*) and cyanobacteria (*Cylindrospermopsis* sp.) were purchased from Carolina Biological Supply Company. Stock unialgal culture tubes were shipped overnight with approximately 10 ml of Alga-Gro freshwater culture medium. Stock cultures were directly used for the bioassay within two hours from their arrival to our laboratory. *Cylindrospermopsis* sp. [54–58] and *Raphidocelis subcapitata* are commonly found in freshwater ecosystems and widely used for bioassays [57]. *Raphidocelis subcapitata* is unicellular autotrophic lunate-shaped microalga containing chlorophylls *a* and b and accessory pigments (such as carotenes and xanthophylls) [58]. *Raphidocelis subcapitata* generally prefers NH_4^+ over NO_3^- as an N source [59]. Additionally, some micronutrients, such as copper, iron, manganese, molybdenum, and zinc, are also required for growth [58,60,61]. *Cylindrospermopsis* sp. exhibits wide tolerance to temperatures ranging from 11 to 35°C, [59,60] with higher temperatures (25–32°C) being favorable for bloom formation [62,63]. *Cylindrospermopsis* sp. is adapted to grow under low light conditions, allowing them to disperse throughout the water column. Additionally, it has been shown to alternate the N requirement between N-fixation [64] and different N sources, including NH_4^+, NO_3^-, and urea [65,66]. Further, *Cylindrospermopsis* sp. has both high uptake affinity and storage capacity for P, which is beneficial when there are fluctuations in P concentrations in aquatic ecosystems [67].

Sterile Falcon 50 ml conical polypropylene centrifuge tubes were used as experimental tubes (vessels) for the bioassay. Each 50 ml treatment, still containing naturally occurring algal communities, was inoculated with 1ml of stock unialgal cultures of *Raphidocelis subcapitata* or *Cylindrospermopsis* sp. for each tube to ensure that all five urban streams received the same test species and an adequate number of algal cells for the bioassay. The initial chlorophyll *a* concentration, or number of algal cells in 1ml of stock unialgal cultures, was not measured as this bioassay was designed to measure biomass changes in treatments relative to the control. Stock cultures, including algae cells and the medium, were used to inoculate both treatments and control tubes. Hence, any P from the culture medium was also transferred into all the treatments and the control. Thus, all the treatments and the control received the same concentration of nutrients from the culture medium. All treatments, including control, were conducted in triplicate.

Following nutrient additions, samples were incubated in a fume hood to reduce dust deposition for seven days under a 16 h light with 8h dark cycle using natural spectrum grow lights [68]. The experimental tubes were shaken daily and left without caps to ensure CO_2 exchange and maintain a stable pH suitable for algal growth. Additionally, sample tubes were moved from left to right and from the middle to the sides of the fume hood daily during the incubation to reduce light bias within the fume hood.

After the seven-day incubation period, the raw absorbance (without extraction of chlorophyll *a*) of the entire algae slurry was measured as a proxy for algal biomass at 678 nm [69] using a Thermo Scientific Evolution 60S UV-visible spectrophotometer to quantify the response of each treatment [67]. While the extraction of chlorophyll *a* is a common method to measure algal biomass, we used an alternative method that measures raw absorbance values as a proxy for algal biomass due to the rapid pace of this project and the large number of samples for each bioassay (288 samples per bioassay). The total biomass changes relative to the control were measured for all treatments at the end of the incubation period. Changes in raw absorbance values are interpreted as relative increases or decreases in primary production [68].

Statistical analyses

Temporal trends in streamwater dissolved solute concentrations and pH were evaluated by aggregating solute concentrations from all five streams for each sampling event throughout the study period. Mean concentrations or pH for each event

were used to visualize trends in streamwater chemistry and Kendall's Tau tests were used to evaluate trend significance. Kendall's Tau is a non-parametric, rank-based correlation coefficient that measures the strength and direction of association between two variables. Kendall's Tau was also used to evaluate temporal trends in WWTP influent and effluent.

For comparison of pre- and post-treatment conditions, we aggregate data across streams (n = 5) and over the spring months of March-April-May to compare median values for spring 2019 and 2020. Wilcoxen-Mann-Whitney tests were used to compare stream pH and solute concentrations in spring 2019 (representing pre-PO_4^{3-} dosing conditions) and 2020 (representing post - PO_4^{3-} dosing conditions) including TDP, TP, TRP, TN, TN:TP, Cu, Mn, Fe, Pb, Mg, B, F^-, K, Si, and Sr. The Wilcoxen-Mann-Whitney test is a non-parametric test used to compare differences between two independent groups and does not assume normality. Not all solutes were measured in spring 2019 and thus were excluded from this analysis (SRP, ^{15}N, NO_3^-). We compared only these three months to minimize seasonal differences across the treatment period, and data from all streams were combined to increase statistical power. May was included in the pre-treatment aggregation based on the rationale that P-dosed drinking water would take several months to appear in urban streams at detectable levels. This is further supported by the fact that although initial treatment by PWSA began on April 1, 2019, not until April 28, 2019, did all seven sites receive concurrent dosing. Due to this staged implementation, as well as the multiple months required for P-based scale build-up in the pipe network [70,71], it is not likely that P-enriched drinking water was dispersed uniformly throughout the distribution network. This time delay is further compounded by the unknown residence time of drinking water in the distribution system pipe network and the transit time required for any drinking water leaks to reach sampled stream reaches through subsurface drainage. We use the same Wilcoxen-Mann-Whitney test to evaluate differences in influent and effluent TP concentrations reported by the WWTP in the PWSA service area for the months spanning pre-treatment (Jan-Feb-Mar-April) in 2019 and post treatment (Jan-Feb-Mar-April) in 2020.

Principal Component Analysis (PCA) was performed to identify the key water quality variables that explain the majority of variance in the dataset and to detect patterns related to the contribution of multiple potential P and other solute sources to urban streams. For the PCA analyses, any sampling dates with missing data were excluded from the analyses resulting in a sample size of 48 that was uniformly spread across study streams and seasons. PCA preserves the maximum amount of variance in the data and is well- suited for identifying the most important data variables. In this process, a dataset with related variables is transformed into a new set of uncorrelated variables known as principal components, where the first two components capture most of the variation in the entire dataset. Data from each watershed were enclosed within shaded polygons intended for visual representation and do not carry any statistical significance.

For the statistical tests described above, one outlier was excluded from analyses that had concentrations of some solutes measured on the ICP-MS (including Cu, Fe, Pb, and Mn) that were higher than the mean solute concentration plus six times the standard deviation. This outlier was a sample collected in Panther Hollow on January 29, 2020. Concentration data for only these solutes were excluded from statistical analyses.

Log-linear regressions between TDP and metals (Cu, Fe, Pb, and Mn) were analyzed after averaging the monthly mean values across five urban stream systems. Metal data were log-transformed after determination of non-normal distributions using the Shapiro-Wilk test.

Differences in the phytoplankton biomass response to experimental nutrient additions (control, + N, + P, + NP) were analyzed using a one-way ANOVA. When significant treatment effects were observed, post hoc multiple comparisons of treatment means were performed using Tukey's least significant difference procedure [72–75]. This test identified whether phytoplankton had a significant nutrient limitation of either N, P, or N and P co-limitation. Normality was tested using the Shapiro-Wilk test, and biomass absorbance data was normally distributed, while nutrient concentrations were normally distributed following log transformations. The mean values and standard deviations of triplicate measurements were used for all statistical tests, using the SPSS 28.0 statistical package. The level of significance used was $p < 0.05$ for all tests.

Results

Phosphate and metal concentrations in the drinking water distribution system

Monthly samples were collected by PWSA from seven sites within their distribution network (Fig 2). Prior to the large-scale PO_4^{3-} additions to the PWSA drinking water distribution system, PO_4^{3-} concentrations were below detection limits on February 12, March 14, and March 29 (Fig 3). PO_4^{3-} additions began on April 1, 2019, whereafter concentrations rapidly increased throughout the distribution system. Once PO_4^{3-} concentrations reached 500 µg P/L, they remained stable throughout the remainder of the study period even after the P dosage was slightly reduced from 1000 µg P/L to 600 µg P/L by September 2019 (Fig 3 and Fig 4). To monitor the impact of the PO_4^{3-} addition on pipe integrity and lead concentrations, PWSA measured pH, PO_4^{3-} concentration, and proxies for pipe corrosion including concentrations of Fe, Cu, Mn, and Pb (Fig 4). Following PO_4^{3-} additions, pH decreased rapidly from 8.4 (April 2019) to 7.6 (September 2019), then rebounded to 7.9 by February 2020 (Fig 4a). Concentrations of Fe and Cu in the drinking water distribution system initially increased following PO_4^{3-} addition, then started to decrease in August and September, respectively (Fig 4c and Fig 4d). Mn concentrations in drinking water decreased sharply upon PO_4^{3-} additions and then rebounded in May 2019 before decreasing again (Fig 4e). Pb concentrations remained elevated until August 2019 after which they decreased sharply until December 2019 and thereafter increased for the remaining duration of the PWSA sampling period (Fig 4f).

Pre-treatment conditions in urban streams

Summary statistics for solute streamwater concentrations in spring 2019, including the months of March, April, and May, are shown in S2 Table. Prior to P-dosing, the median spring (2019) TDP, TP, and TRP concentrations across all

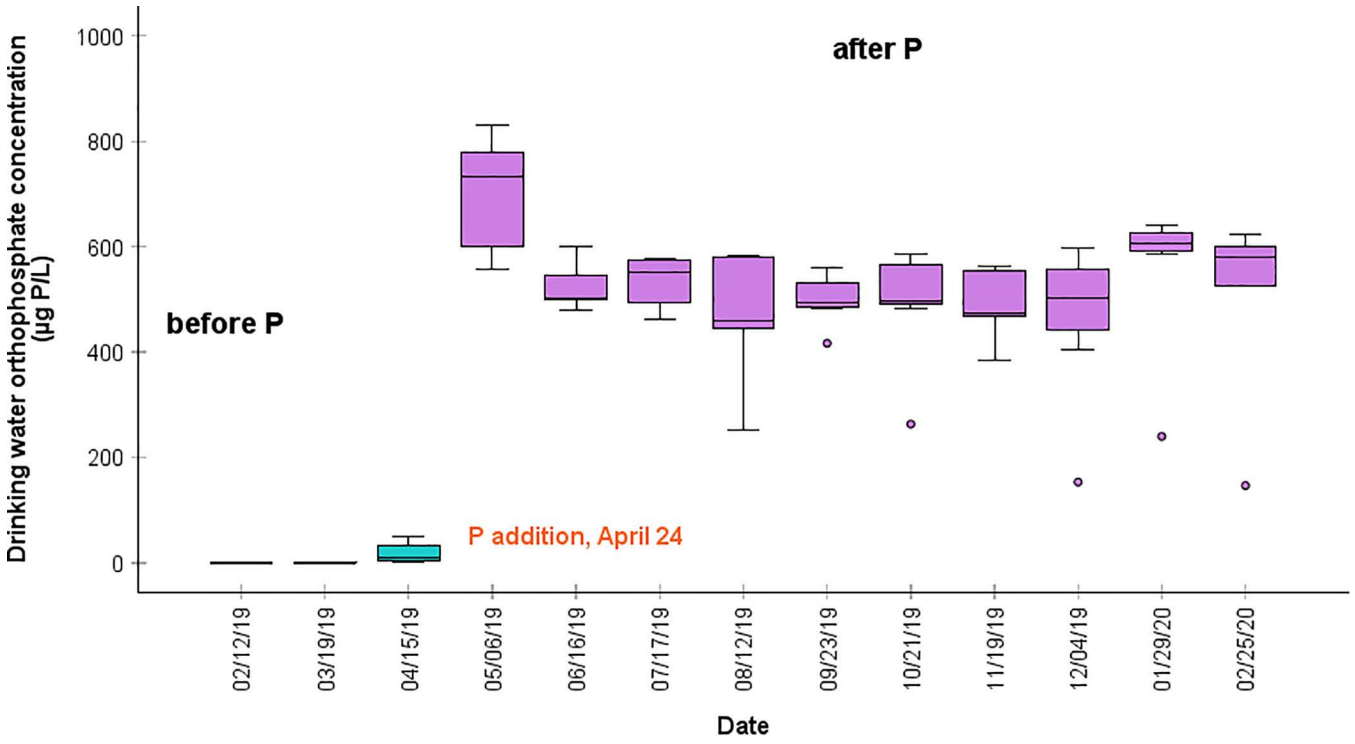


Fig 3. Boxplots showing median and outliers (circles) for PO_4^{3-} concentration across the seven drinking water distribution system sites collected by Pittsburgh Water and Sewer Authority (PWSA) before (Feb-April 2019) and after (May 2019 to Feb 2020) PO_4^{3-} addition.

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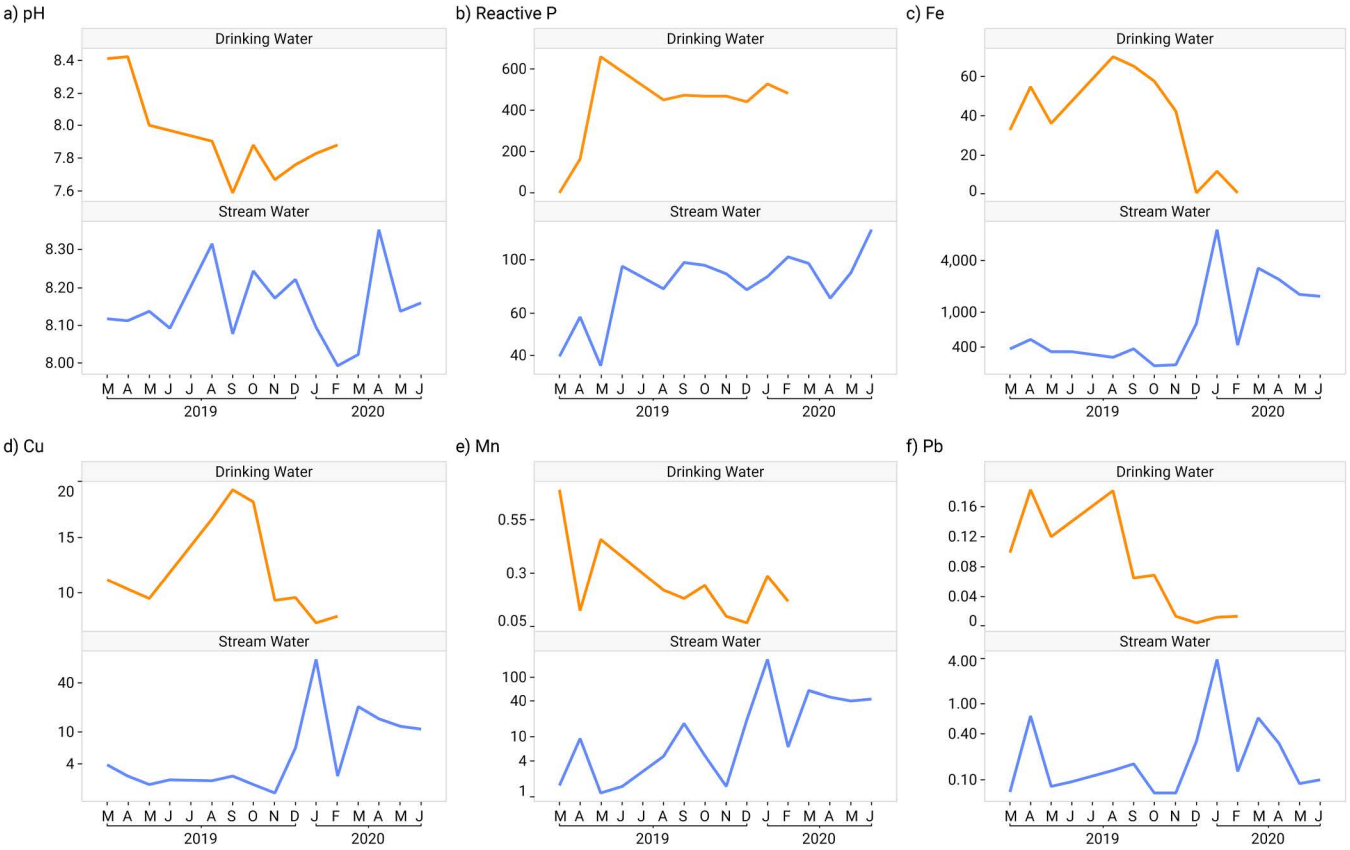


Fig 4. Drinking water distribution system (DW) chemistry sampled from seven locations in the pipe network are shown in orange including (a) pH and concentrations of dissolved (b) soluble reactive phosphorus (SRP), (c) iron (Fe), (d) Copper (Cu), (e) Manganese (Mn), and (f) Lead (Pb) for the study period. For comparison, urban stream chemistry for the same dissolved solutes is shown in blue including concentrations of (b) total reactive phosphorus (TRP). Mean stream chemistry is aggregated across five urban streams and for each sampling event throughout the study period. All solute concentrations are reported in μg/L.

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five streams were 24.5 μg P/L, 31.5 μg P/L, and 25.7 μg P/L, respectively (Fig 5a-5c, S2 Table). Across the five stream systems, pre-treatment streamwater concentrations of TDP, TP, and TRP were variable, but for all P species quantified, the highest median concentrations prior to treatment were observed in Negley Run (S1 Fig). Median spring stream TP concentrations exceeded the 25th percentile median TP concentrations (12 μg P/L) observed in streams and rivers in Ecoregion XI in Pennsylvania [37] indicating the impaired conditions in these urban streams. Concurrently, median solute concentrations of Mn, Cu, Fe, and Pb were 1.3 μg/L, 3.3 μg/L, 368 μg/L, and 0.1 μg/L, respectively (Fig 5e-5h, S2Table). In comparison, median concentrations during this same period of solutes indicative of weathering, including Si, Sr, K, and Mg were 3,935 μg/L, 389 μg/L, 2,665 μg/L and 18,900 μg/L, respectively (Fig 5i-5l). During this same period, median concentrations of solutes that are typical tracers of wastewater, including TN, F^-, B, and TN:TP were 1490 μg/L, 203 μg/L, 53 μg/L and 84, respectively (Fig 5m-5p).

Post-treatment conditions in urban streams

After the initiation of P additions on April 1, 2019, mean concentrations of TDP across all streams had a highly significant increasing trend (Kendall's Tau = 0.72, $p < 0.0001$) (S2a Fig). Mean concentrations of TDP in streams increased from 50 μg P/L in March 2019–269 μg P/L in Jun 2020 (S2a Fig). Additionally, increasing trends in TP and TRP were also significant

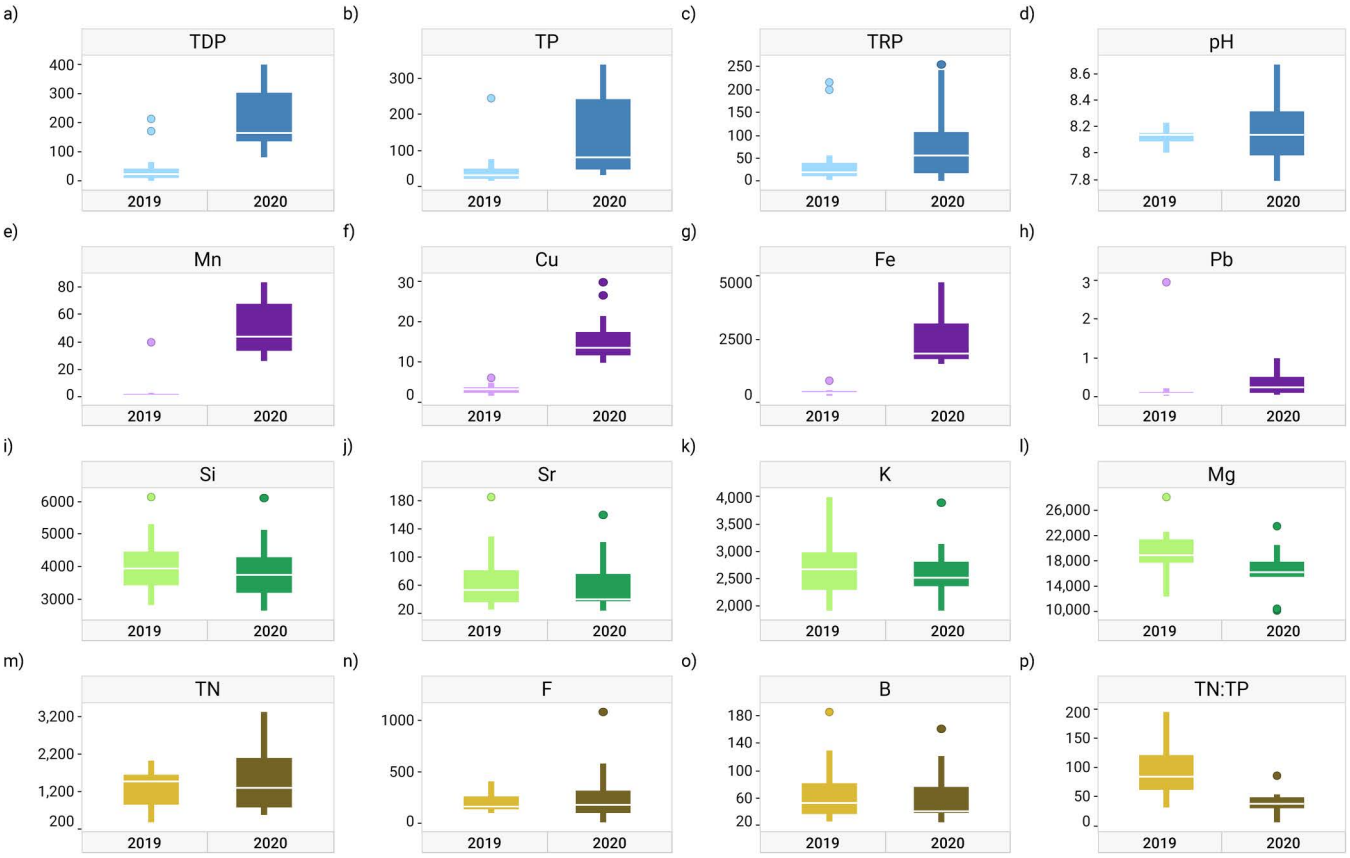


Fig 5. Comparison of urban stream chemistry from five watersheds prior to P additions to drinking water (spring 2019) and one year after P treatments began (spring 2020) where spring months comprise March-April-May. For all panels, solute concentrations are reported in μg/L. Panels a-d (blue) represent major forms of P collected across the study period including (a) total dissolved phosphorus (TDP), (b) total phosphorus (TP), (c) total reactive phosphorus (TRP), and (d) pH. Panels e-h (purple) include metals monitored by the DWTP as a proxy of pipe corrosion including (e) manganese (Mn), (f) copper (Cu), (g) iron (Fe), and (h) lead (Pb). Panels i-l (green) include solutes commonly used to indicate weathering of soils and bedrock including (i) silica (Si), (j) strontium (Sr), (k) potassium (K), and (l) magnesium (Mg). Panels m-p (yellow) include concentrations of wastewater tracers including (m) total nitrogen (TN), (n) fluoride (F^-), boron (B), and (p) ratios of total nitrogen to total phosphorus. Observed concentration increases of TDP, Cu, Fe, Mn from 2019 to 2020 were highly significant ($p < 0.0001$) whereas increases TP and Pb over the same time period were also significant ($p < 0.005$). From 2019 to 2020, concentrations of Mg significantly decreased ($p < 0.05$), as well as TN:TP ($p < 0.005$).

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(Kendall's Tau = 0.43 and 0.38, respectively, $p < 0.05$) (S2b and S2c Fig). The increasing trend in TP concentrations over the study period caused a general decrease in the ratios of TN to TP (TN: TP) but the trend was not significant (S2d Fig).

Similar to TDP, TP, and TRP, concentrations of Mn concentrations had a highly significant increasing trend (Kendall's Tau = 0.60, $p < 0.0001$, not shown). Although concentrations of Fe, Cu, and Pb increased, and pH decreased, the trends were not significant over the study period (not shown).

From spring 2019 to spring 2020, Wilcoxon-Mann-Whitney tests indicate highly significant increases in concentrations of TDP ($p < 0.0001$) where median concentrations increased by 614% and significant increases in TP concentrations ($p < 0.005$) where median concentrations increased 105% (Fig 5a-5b, S2 Table). There was no statistically significant increase in TRP concentrations or pH between the spring months of 2019 and 2020 (Fig 5c-5d, S2 Table).

Meanwhile, streamwater concentrations of metals used by the DWTP to indicate pipe corrosion increased significantly in streamwater from spring 2019–2020 including highly significant increases in Mn ($p < 0.0001$, 3,481% increase), Cu ($p < 0.0001$, 317% increase), and Fe ($p < 0.0001$, 462% increase), although there was no statistically significant increase in

Pb concentrations (Fig 5e-5h, S2 Table). In contrast, median concentrations of solutes indicative of weathering products including Si, Sr, and K, were not significantly different from spring 2019 to spring 2020 (Fig 5i-5l) whereas median Mg concentrations significantly decreased between spring 2019 and 2020 ($p < 0.05$) (Fig 5i-5l, S2 Table). Likewise, solutes indicative of contributions of wastewater to urban streams including TN, F^-, and B did not significantly increase from spring 2019 to spring 2020 (Fig 5m-5o) [76]. TN:TP ratios decreased significantly between 2019 and 2020 (<0.005) due to the significant increase in TP values (Fig 5p, S2 Table).

Algal response to nutrient addition bioassay

Nutrient addition bioassay of phosphate-treated tap water. *Cylindrospermopsis* sp. (cyanobacteria) and *Raphidocelis subcapitata* (green algae) biomass response to +N, + P, and +NP treatments before and after PO_4^{3-} addition to drinking water distribution network, are shown in Fig 6. Two months following PO_4^{3-} additions, *Cylindrospermopsis* sp. biomass did not significantly increase ($p < 0.05$) in any treatments relative to the tap water control (Fig 6a, S3 Table). However, *Raphidocelis subcapitata* biomass significantly increased ($p < 0.05$) two months following PO_4^{3-} additions for +NP treatments relative to the tap water control (Fig 6b, S4 Table). However, twelve months after PO_4^{3-} addition, only the + 2N caused a significant increase ($p < 0.05$) while the response of +5N was inconclusive for *Cylindrospermopsis* sp. biomass in tap water (Fig 6, S3 Table). After 12 months, *Raphidocelis subcapitata* biomass significantly increased with both +N alone and +NP treatments (Fig 6, S4 Table).

Nutrient addition bioassay of phosphate-treated stream water. In streamwater, the *Cylindrospermopsis* sp. and *Raphidocelis subcapitata* biomass response to +N, + P, and +NP treatments were examined at three time intervals: prior to PO_4^{3-} addition, two months, and twelve months following PO_4^{3-} addition across all streams. Aggregated results across all streams are shown in Fig 7. Following two and twelve months of P additions to the drinking water distribution network, *Cylindrospermopsis* sp. did not exhibit a significant ($p < 0.05$) increase in response to any treatment compared to the streamwater control (S3 Table). Nevertheless, when compared to the pre-addition period, *Cylindrospermopsis* sp. biomass increased significantly ($p < 0.05$) with +N two months following P addition. However, this effect was not sustained, as no significant difference was observed between the pre-addition period and twelve months post-addition. While no significant biomass increase was detected with +P at either period, *Cylindrospermopsis* sp. biomass increased significantly ($p < 0.05$) with +NP two months after P addition compared to pre-P addition. However, no significant ($p < 0.05$) biomass increase was detected with +N twelve months following P addition compared to the pre-addition period (Fig 7a; S3 Table).

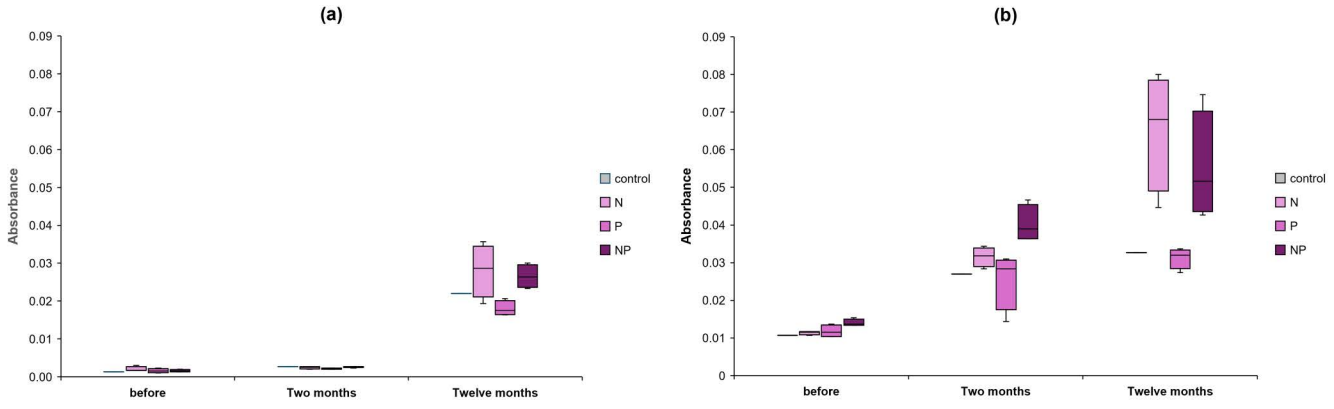


Fig 6. Mean tap water response of (a) *Cylindrospermopsis* sp. (cyanobacteria) and (b) *Raphidocelis subcapitata* (green algae) (as absorbance at 678 nm, a proxy for algal biomass), + P, + N, or +NP additions before, two months after, and twelve months after PO_4^{3-} addition to tap water.

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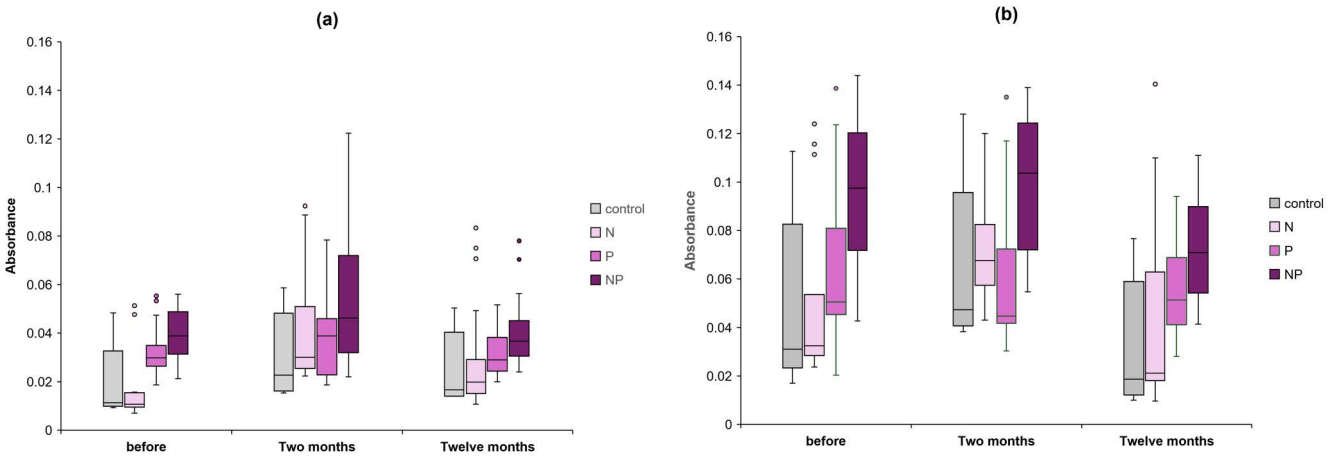


Fig 7. Mean streamwater response of (a) *Cylindrospermopsis* sp. (cyanobacteria) and (b) *Raphidocelis subcapitata* (green algae) (as absorbance at 678 nm, a proxy for algal biomass) to streamwater control, + P, + N, or +NP additions before, two months after, and twelve months after PO_4^{3-} addition to drinking water.

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In response to treatments across three periods, *Raphidocelis subcapitata* biomass increased significantly ($p < 0.05$) with +NP compared to +N and +P (Fig 7b, S4 Table). However, no significant increase was observed with any treatment when compared to the streamwater control. When comparing biomass changes over time, *Raphidocelis subcapitata* biomass differed significantly ($p < 0.05$) between two months and twelve months following PO_4^{3-} addition.

Individual stream responses varied. Prior to PO_4^{3-} addition to the drinking water distribution network, *Cylindrospermopsis* sp. biomass in Negley did not show a significant increase ($p < 0.05$) under any treatment (+N, + P, or +NP) compared to the streamwater control (S3 Table). However, *Cylindrospermopsis* sp. biomass in the other four streams (Shades, Fern, Phipps, and Panther) increased significantly ($p < 0.05$) with P and NP additions compared to N addition (S3 Table). Similar to *Cylindrospermopsis* sp., no differences in *Raphidocelis subcapitata* biomass were observed in Negley across the three time periods; nevertheless, the other four streams showed variable responses between the pre-addition period and two months post-addition, as well as between the pre-addition period and twelve months post-addition (Fig 7b, S4 Table).

Discussion

Changes in drinking water pipe network chemistry

Following the initiation of PO_4^{3-} additions by PWSA, pH concentrations in the drinking water distribution network decreased quickly (Fig 4a-4b). Meanwhile, concentrations of corrosion products from iron pipes, such as Fe, Cu, and Pb increased in the drinking water pipe network, while concentrations of Mn decreased, albeit at different times (Fig 4c-4f). While Fe and Pb concentrations increased quickly following treatment, concentrations of Cu peaked later in September 2019 (Fig 4c-4d). Pb concentrations remained high in the pipe network until August 2019, after which they declined until December 2019 (Fig 4f). These temporal shifts in concentrations reflect the process of destabilization of the pipe interior caused by lower pH following the PO_4^{3-} addition (Fig 4a-4b). Studies have shown that introducing PO_4^{3-} can initially increase Pb and other metals due to the disturbance of pre-existing scales, particularly in systems with high alkalinity [77]. This temporarily increases the solubility of lead-based corrosion products and gradually decreases as a new PO_4^{3-} -based layer is formed [12].

While PO_4^{3-} helps form a stable lead PO_4^{3-} layer that effectively prevents further metal corrosion, the process takes time [71]. Studies indicate that stabilization can take anywhere from a few weeks to several months, depending on factors such as water chemistry, pipe age, and initial scale composition [71]. A study by Knowles et al. [70] found that in some systems,

Pb concentrations began to decline significantly after 3–6 weeks, while in others, it took up to 6 months for full stabilization. In a case study from Washington, D.C., the implementation of PO_4^{3-} treatment resulted in a gradual decline in Pb levels over a period of several months, with notable improvements observed after 6 months to a year [77]. Once equilibrium is reached, PO_4^{3-} significantly reduces Pb corrosion by maintaining a stable, low-solubility protective layer on the pipe's inner surface. In this study, data collected by PWSA suggests that by August 2019, approximately three months following PO_4^{3-} treatment, a new PO_4^{3-} scale had formed. This is evidenced by the onset of declining Pb concentrations, a trend that persisted through December 2019 (Fig 4f).

Drinking water pipe network interactions with stream chemistry

A comparison of pre- and post-treatment stream chemistry for the months of March-April-May in 2019 and 2020 revealed significant increases in median concentrations of TDP and TP representing increases of over 600% and 100%, respectively (Fig 5a-5b, S2 Table). Meanwhile, streamwater concentrations of metals used as proxies of pipe corrosion including Cu, Mn, Fe, and Pb also increased significantly by over 300%, 3,400%, 400%, and 200%, respectively (Fig 5e-5h, S2 Table). Together, these findings provide compelling evidence that the additional dissolved P and TP observed across the stream systems originates from the drinking water pipe network. Given that this comparison spans equivalent spring months during two study years, it also indicates that the differences we document are not attributable to seasonal effects on P concentrations. These findings reveal that sufficient subsurface connectivity exists to allow leaks from the drinking water pipe network, characterized by high concentrations of PO_4^{3-} and treatment-induced disruption of the existing pipe scale, measurably altered urban stream chemistry. Specifically, these leaks resulted in discernable and significant increases in TDP, TP, Cu, Fe, Mn, and Pb.

The attribution of the dissolved P and TP from drinking water pipe network leaks is further supported by the fact that a similar comparison using a Wilcoxon-Mann-Whitney test found no significant difference in concentrations of typical wastewater indicators, including F^-, TN, or B concentrations, between spring months of 2019 and 2020 (Fig 5m-5p, S2 Table). Like many water providers, PWSA adds F^- to drinking water to protect public health, however, the F^- treatment did not change between spring of 2019 and 2020 [78,79] (Fig 5n, S2 Table). Similarly, TN that could potentially be sourced from sewer system leaks, fertilizer, or soil N cycling, was not significantly different between spring 2019 and 2020 (Fig 5m, S2 Table). Importantly, because of the relative increases in TP, and the fact that TN did not change significantly, TN:TP significantly decreased by 54% between spring 2019 and 2020 ($p < 0.001$) (Fig 5p, S2 Table).

Studies have shown that *Cylindrospermopsis* sp. can be an opportunistic species due to its ability to utilize a wide range of dissolved P, its high uptake affinity, and its large storage capacity for P [80]. These characteristics provide a significant advantage, even when P concentrations fluctuate. Nevertheless, *Cylindrospermopsis* sp. tends to dominate when the TN: TP ratio is either very low or very high [81]. Although the Redfield ratio of 16:1 does not necessarily predict limiting nutrients [82,83], extreme ratios can limit growth [84]. We observed that the median spring streamwater TN:TP ratios decreased from 84 in 2019 to 39 in 2020, which further highlights the importance of P availability in regulating abundance of bloom forming *Cylindrospermopsis* sp. in nutrient-rich urban stream systems.

Together, these significant differences between equivalent seasons in different years suggest several major controls on urban stream solute chemistry. We explore solute relationships further using a PCA (Fig 8). Two principal components (PCs) cumulatively explain 54.5% of the variance in stream solute concentrations. A significant portion of variance (30.7%) in stream solute chemistry is explained by PC1. Mg (Magnesium) has the largest negative loading (-0.421), along with negative loadings in Ca (Calcium, -0.359), Sr (Strontium, -0.343) and Si (Silicon, -0.365). The negative loadings indicate that these mineral elements share a similar trend and decrease along with PC1. On the other hand, TP has a moderate positive loading (0.244), indicating a contrasting trend to the negatively loaded elements. Similarly, TDP shows a weaker positive loading (0.169), reinforcing this distinction. Together, these loadings imply that PC1 reflects a contrast between mineral elements (e.g., Mg, Ca, Sr, and Si) and nutrient compounds (e.g., TP and TDP). The positive loadings of TP, TDP,

and TN on PC1 align closely with sewage sources and suggest nutrient enrichment likely originating from wastewater discharges. In contrast, the negative loadings of Si, Mg, K, and Sr on PC1 reinforce a divide between natural geochemical inputs and human-driven pollution.

PC2 represents a secondary dimension of variation, explaining an additional 23.8% of solute variability and highlights the role of trace elements (Fig 8). Cu (Copper) has the strongest positive loading (0.487), making it a dominant factor in shaping PC2. This is closely followed by Fe (Iron, 0.479) and Mn (Manganese, 0.421), all of which contribute significantly to this component. This indicates that PC2 primarily captures variation driven by trace metals (e.g., Cu, Fe, and Mn) that may result from pipe corrosion.

The PCA biplot provides insights into how streamwater samples from the five study watersheds differ based on their chemical composition (Fig 8). Phipps Run streamwater samples appear to be the most impacted by elements aligned with PC2 typical of iron pipe corrosion including Mn, Pb, Fe, and Cu. In comparison, Fern Hollow and Shades Run samples group closer to the negative side of PC1, indicating higher concentrations of minerals like Si, Mg, and Sr and lesser influences from wastewater and pipe corrosion. Conversely, samples from locations such as Negley Run and Panther Hollow

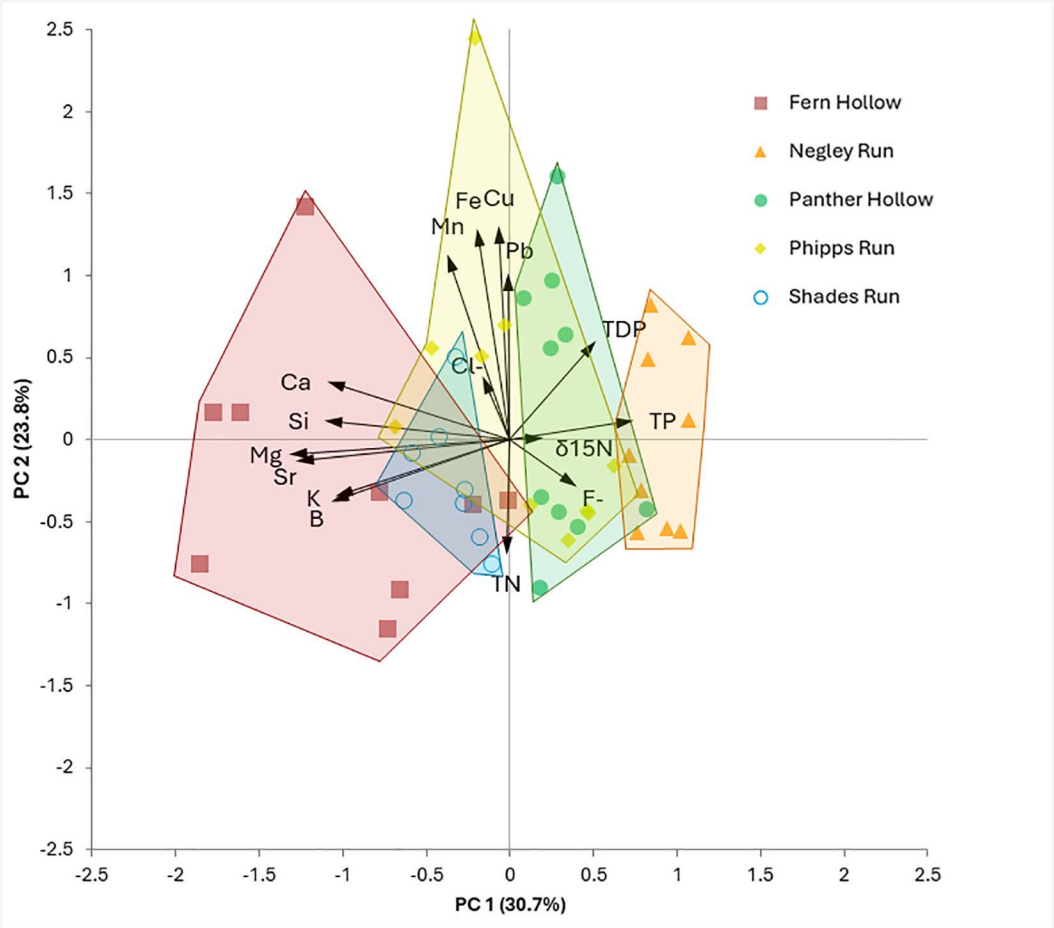


Fig 8. Principal component analysis (PCA) for concentrations of total dissolved phosphorus (TDP), total phosphorus (TP), total nitrogen (TN), metals, and $\delta^{15}N\text{-}NO_3^-$ values in five urban streams over the study period (March 2019 to June 2020). The first two components explained 30.7% and 23.8% of the variances, respectively. Arrows indicate the contribution of each variable to the first two PCs. Colored polygons bracket samples from individual watersheds shown in a corresponding color.

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cluster closer to the positive side of PC1, suggesting higher levels of TP, TDP, and higher $\delta^{15}N\text{-}NO_3^-$ values. Together, the PCA results suggest that Fern Hollow and Shades Run are mineral-dominated, while Negley Run and Panther Hollow are nutrient-enriched from wastewater, whereas Phipps Run may be the most impacted by corrosion byproducts.

Dissolved P and metal complexes

The findings reported here indicate that the addition of PO_4^{3-} initially increased dissolution of iron oxyhydroxides in pipe minerals or scale and mobilized Cu, Fe, Mn, and Pb (Fig 4b-4f). Dissolved concentrations of these same elements increased across the five urban streams included in this study (Fig 4b-4f). The timing of the increase in streamwater metal concentrations coincides with the increase in streamwater TDP such that strong and significant, linear correlations exist between TDP and log transformed metals including Mn ($R^2 = 0.73$, $p < 0.0001$), Cu ($R^2 = 0.72$, $p < 0.0001$), and Fe ($R^2 = 0.70$, $p < 0.0001$). Additionally, there is a significant, though weaker, correlation between TDP and Pb ($R^2 = 0.23$, $p < 0.005$) (Fig 9).

The strong associations between TDP and metal ions such as Cu, Pb, Fe, and Mn are unlikely to be coincidental, given the potential formation of phosphate-metal complexes. These associations suggest two primary sources of metal-phosphate complexes in the urban environment. First, PO_4^{3-} leaked from the drinking water distribution system may enter subsurface soils or groundwater where it can interact with legacy metals during transport to the stream. Pittsburgh's industrial history has distributed metals across the landscape through steel and coke production, with atmospheric deposition leading to elevated concentrations in soils and groundwater [85]. For example, Mullins and Bain [86] documented elevated concentrations of steel-associated metals including Cobalt (Co), Vanadium (V), Nickel (Ni), and Chromium (Cr), at groundwater discharge points. Additionally, slag, which is a by-product of steel production that often contains calcium phosphate compounds, was widely dumped throughout urban Pittsburgh [87,88]. These legacy materials may continue to influence metal and P dynamics in urban streams, potentially resulting in spatially variable P-metal associations.

To explore this option, we evaluated relationships between TDP and metal ions (Cu, Fe, Pb, Mn) within each of the five study watersheds (S3 Fig). Results showed consistent, positive associations across all watersheds and metals, suggesting that spatial heterogeneity in legacy metal patterns is unlikely to be the primary sources of the observed co-occurrence.

A second, and more likely, explanation is that metals and TDP co-originate from corrosion of drinking water infrastructure. This would account for the consistent, positive associations between TDP, and metals observed across all streams during the study period, supporting the idea that they are traveling together from a common source. Despite current limitations, the data substantiates a coherent framework for understanding the dynamics behind this co-occurrence. While the precise mechanisms remain unresolved, the topic is clearly ripe for future investigation with important implications for ecosystem function, water quality, and human health.

Nutrient addition bioassays

Impacts of phosphate addition on algal growth in tap water. In freshwater ecosystems, P functions as a primary limiting nutrient [18,19,37]. Stream and river ecosystems have complex biogeochemistry that involves strong terrestrial connections, hydrological changes, watershed characteristics, and unidirectional flow, all of which can highly influence nutrient limitation in receiving waters [16,17,19] bioassay results document changing nutrient dyanmics in tapwater and streamwater. Nutrient additions to tap water samples showed that *Cylindrospermopsis* sp. has higher N and P requirements than *Raphidocelis subcapitata*. Although PO_4^{3-} concentrations were below the detection limit in tap water before the PO_4^{3-} addition (Fig 3), *Raphidocelis subcapitata* biomass significantly increased with the highest NP addition (5 mg N/L, 0.5 mg P/L) (Fig 6 and 10; S4 Table). In contrast, *Cylindrospermopsis* sp. biomass did not respond to any N or P additions before P-additions and two months after P-additions (S3 Table). However, biomass significantly increased ($p < 0.05$) with +N twelve months after P-addition (S3 Table). This may be attributed to the high TN:TP requirement of

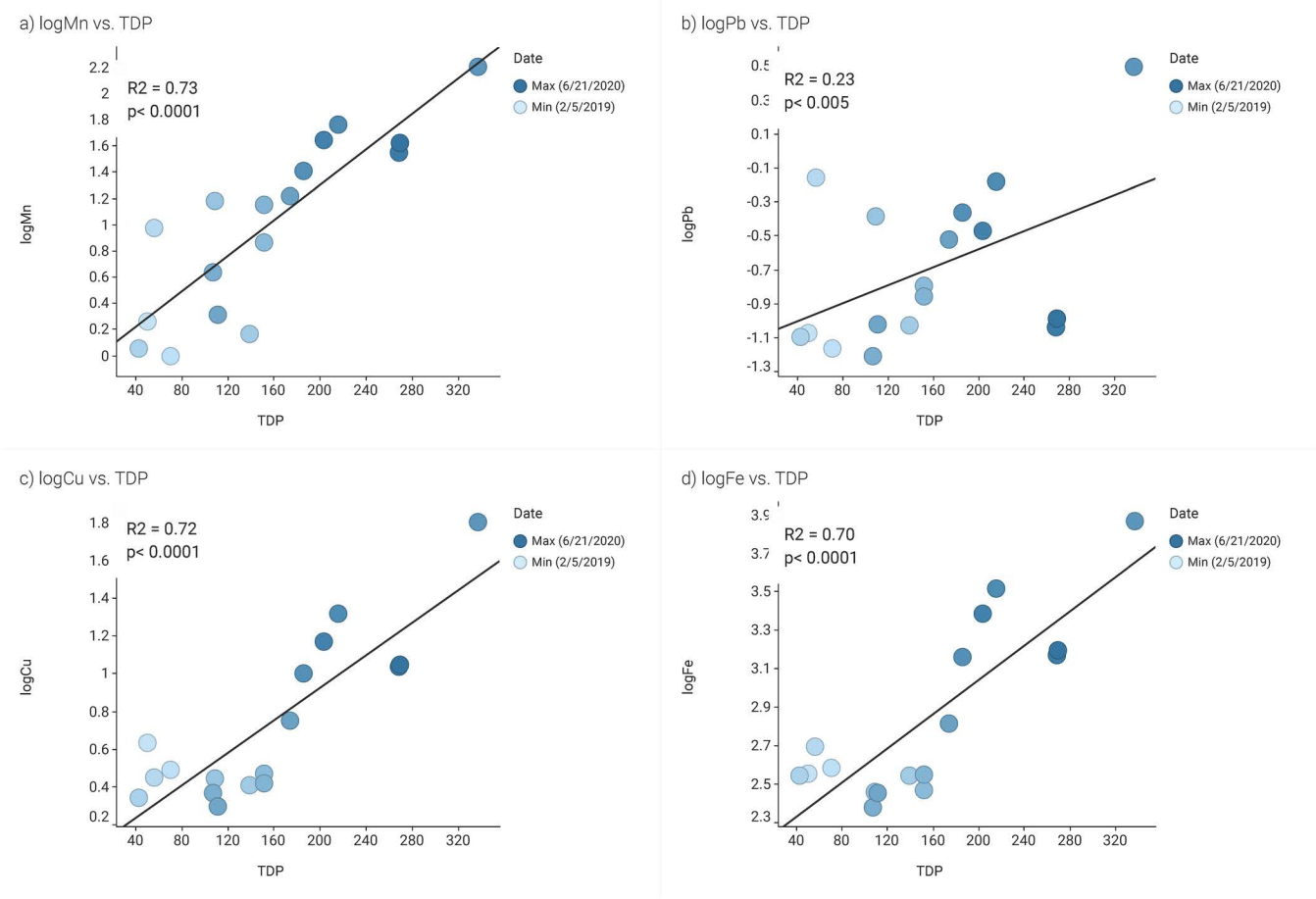


Fig 9. Log-linear regressions of streamwater concentrations of TDP with (a) Manganese (Mn), (b) Lead (Pb), (c) Copper (Cu), and (d) Iron (Fe). Concentrations are mean values averaged for each sampling event across five urban stream systems. TDP concentrations are strongly and significantly correlated with Mn, Cu, and Fe ($p < 0.0001$) and significantly, but more weakly, correlated with Pb concentrations ($p < 0.005$).

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Cylindrospermopsis sp. [89]. These results are compatible with a companion study, which quantified the cyanobacteria population using droplet digital PCR (ddPCR) targeting the 16S rRNA genes and observed that cyanobacteria abundance decreased in the drinking water distribution system following PO_4^{3-} treatment [90–92]. Meanwhile, no statistical change in cyanobacteria abundance was observed in another companion study that evaluated microbial community shifts in the same streams reported here [93].

Impacts of phosphate addition on algal growth in urban stream water. Of the five urban stream systems, only the most eutrophic stream, Negley, showed no limitation towards N and P, suggesting that existing N and P concentrations were high enough to not limit either *Raphidocelis subcapitata* or *Cylindrospermopsis* sp. (S5 Table). Additionally, we determined that combined enrichment with +NP led to higher *Raphidocelis subcapitata* and *Cylindrospermopsis* sp. biomass than +N or +P alone in four of the five headwater urban streams examined (Fig 10, S3 Table, S4 Table). These findings are similar to prior studies that observed co-limitation following combined N and P additions in microcosm and mesocosm nutrient addition bioassays and promoted *Cylindrospermopsis* sp. and *Raphidocelis subcapitata* growth [73,94]. Together, these findings further highlight the need for dual nutrient reductions to help mitigate *Cylindrospermopsis* sp. blooms in freshwater lakes and downstream ecosystems [95–98] and to improve water quality in receiving urban water

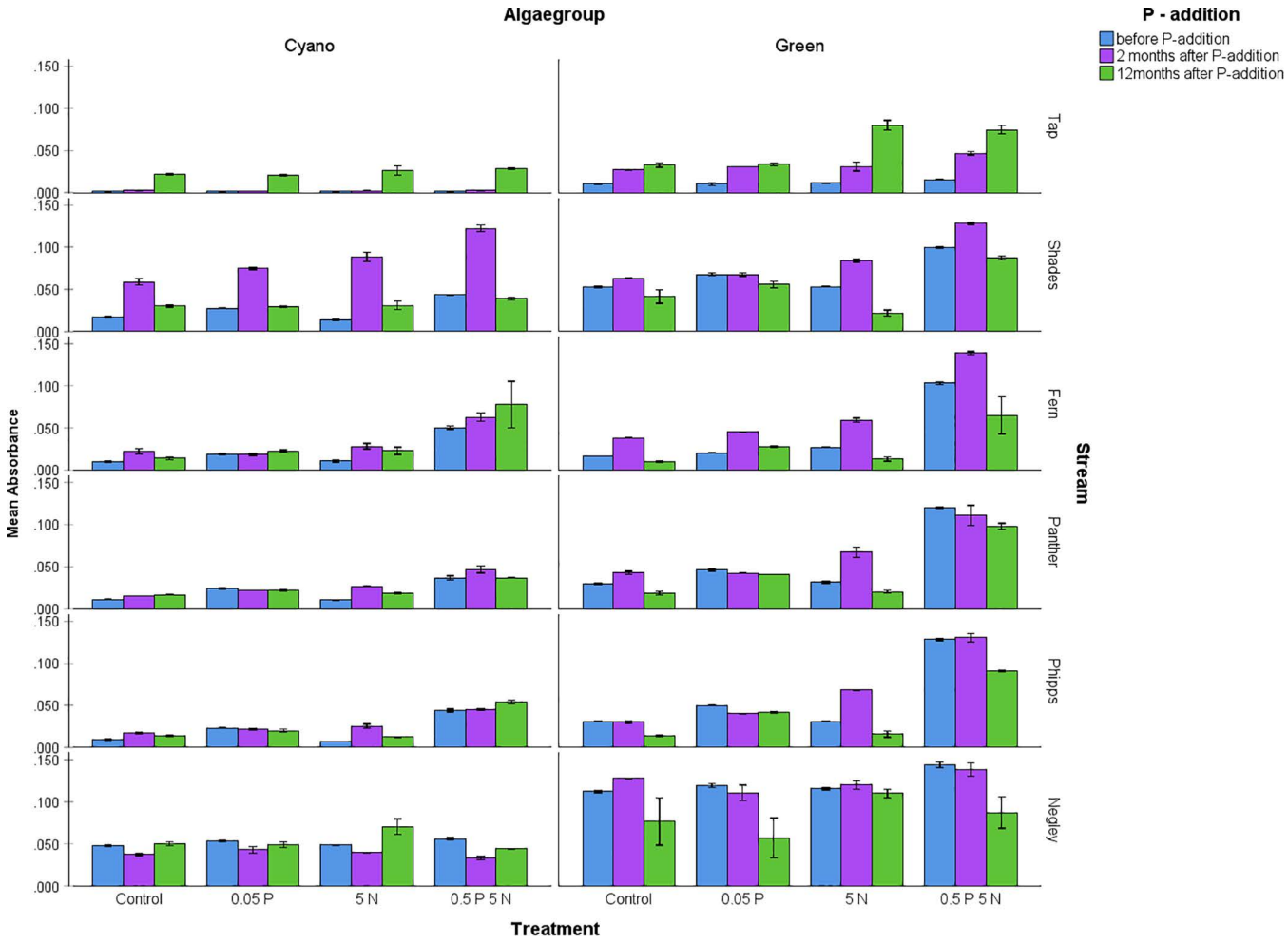


Fig 10. Mean response of *Cylindrospermopsis* sp.(cyanobacteria: "Cyano") and *Raphidocelis subcapitata* (green algae: "Green") (as absorbance at 678 nm, a proxy for algal biomass) to control (that was enriched with phosphate for the two post-treatment assays), + P (0.05P), + N (5N), or +NP (0.5P5N) additions before (blue), two months after (purple), and twelve months (green) after PO_4^{3-} addition in five streams (Panther, Fern, Negley, Phipps, Shades) and tap water (Tap).

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bodies [52]. Importantly, these results underscore that urban streams, impacted by PO_4^{3-} treated drinking water leaks or sewer leaks, can create prime conditions for accelerated eutrophication from the increased availability of both N and P.

We observed different responses from *Cylindrospermopsis* sp. and *Raphidocelis subcapitata* to nutrient additions. Numerous factors can govern the growth difference between *Cylindrospermopsis* sp. and *Raphidocelis subcapitata.* In particular, studies have shown that *Cylindrospermopsis* sp. has high excess P [67,99] and N storage capacity [100]. Thus, *Cylindrospermopsis* sp. may have an advantage over *Raphidocelis subcapitata* when N and P are limited; for example, *Cylindrospermopsis* sp. is capable of utilizing organic P sources in addition to inorganic P as the sole P source when inorganic P is limited in the environment [101] and is also able to dominate in a very low, as well as very high TN:TP conditions [81]. Additionally, we observed higher concentrations of trace metals, including Fe and Cu, during the study period and a significantly increasing trend in Mn concentrations (Kendall's Tau = 0.60, $p < 0.0001$) following P addition to urban streams. Compared to the pre-addition period in spring 2019, concentrations of all three trace metals were significantly higher in spring 2020 following P addition ($p < 0.0001$) (Fig 5e–5g). While Fe and Mn are essential trace metals for

photosynthesis in both *Cylindrospermopsis* sp. and *Raphidocelis subcapitata* [102,103], Cu can be toxic at elevated levels [104,105]. However, trace metal requirements vary widely among algal species, and the combined effects of these metals on specific taxa remain uncertain.

We also observed that both *Cylindrospermopsis* sp. and *Raphidocelis subcapitata* had significantly higher productivity two months after drinking water PO_4^{3-} addition than after twelve months in Shades Run across all treatments. In Fern Hollow, biomass significantly increased at two months, but there was no significant difference between two and twelve months. Although the other three streams (Panther Hollow, Phipps Run and Negley Run) showed no overall significant increases, biomass of both *Cylindrospermopsis* sp. and *Raphidocelis subcapitata* increased in some treatments (Fig 10, S3 Table, S4 Table). Between the time intervals for the two- and 12-month bioassays, spring streamwater concentrations of TDP increased by 614% (Fig 5a) suggesting two-month productivity was catalyzed by drinking water PO_4^{3-} enrichment. Because TN concentrations did not increase over the study period (Fig 5m), the resulting TN:TP ratio in streamwater decreased in 2020 (Fig 5p). It is possible that the decrease in productivity observed for some treatments in three stream systems (Panther Hollow, Phipps Run and Negley Run) for the 12-month bioassay may be due to increasing N limitation relative to PO_4^{3-} enrichment or another factor limiting growth.

To our knowledge, our study is the first to evaluate the combined effects of both seasonal urban stream chemistry and drinking water PO_4^{3-} addition on *Cylindrospermopsis* sp. and *Raphidocelis subcapitata.* Our findings indicate that (1) the P threshold for *Cylindrospermopsis* sp. growth is higher than for *Raphidocelis subcapitata* in the study streams and (2) PO_4^{3-} addition caused a temporary shift in nutrient limitation in *Raphidocelis subcapitata* in Fern Hollow and Panther Hollow among the five studied streams. These shifts in nutrient limitation were accompanied by significant changes in microbial community composition in five studied urban streams after P dosing [91,92]. For example, PO_4^{3-} addition, significantly increased the relative abundance of Actinobacteria, Planctomycetes, and Chlamydiae, which are previously linked to P uptake in soils and marine environments [106–108]. Notably, cyanobacterial abundance remained unchanged [91]. These findings, along with our bioassay, further indicate that different microbial communities have varied P requirements, and it is important to continue to monitor the long-term impacts of P dosing on microbial communities, including *Cylindrospermopsis* sp., in these urban streams.

Although we observed significant changes in phytoplankton biomass during bioassay experiments, there are a few caveats to using bioassay experiments under laboratory conditions. Generally, bioassay experiments cannot account for nutrient recycling and sediment-water interactions that can impact phytoplankton productivity [109]. For example, in bioassays, phytoplankton has a finite supply of nutrients in a closed system, and nutrient concentrations decline with algal uptake, limiting further growth. Another caveat is that, although widely used, bottle-based bioassay experiments are subject to "bottle effects" that can skew nutrient demand due to the exhaustion of nutrient supplies in enclosed containers [110]. Bottle effects can interfere with findings, especially with long bioassays (i.e., seven days or more) and the use of small vessels. Additionally, we inoculated algae directly from the liquid growth medium; hence, algae were not starved by either P or N at the start of the bioassay. *Cylindrospermopsis* sp. is a known N-fixing cyanobacterium, and N-fixation occurs only when N is limited and significantly increases at higher P concentrations [111]. However, in our bioassay, we did not observe an increase in *Cylindrospermopsis* sp. biomass under N or P-deficiency conditions compared to *Raphidocelis subcapitata* biomass. We observed N deficiency in some urban streams occasionally; hence, we do not believe N-fixation interfered with our findings. Additionally, our N treatments may have also increased potassium concentrations through the addition of KNO_3. A previous study showed the importance of potassium as a cofactor and osmolyte in algal growth; however, exceeding the required amount of potassium may depress growth, reduce algal biomass, and interfere with the findings [112]. Lastly, in contrast to bioassays, under field conditions, phytoplankton growth rates are either balanced or exceeded due to internal and external nutrient sources. Thus, the same nutrient might not be a limiting nutrient in bioassay experiments as in field conditions. To address these concerns, we collected and analyzed field water samples from the same streams during the same time of year to interpret our bioassay in the context of collected water chemistry.

With these caveats in mind, we interpret changes in the relative absorbance of algal biomass and a relative increase or decrease in primary production.

Potential confounding factors

Pre-treatment sampling was limited due to the rapid pace of PWSA action and delayed project funding. Due to this limitation, as well as a relatively small number of stream study sites, we opted to aggregate our results across the five stream systems. Additionally, to compare pre- and post-treatment changes, we aggregated data into multiple months, including March, April, and May. While both approaches yield an overall improvement in statistical power, more pre-treatment sampling would have been ideal and would have facilitated more in-depth explorations of changes within individual watersheds. Additionally, our field observations were limited to five stream reaches, as most Pittsburgh streams are buried in sewer networks [32–33]. Lastly, we cannot conclusively determine whether drinking water pipes were located directly upstream of our sampling sites and if so, how far from the drinking water pipe to our urban stream sampling sites. While this would have been useful, all stream monitoring sites were located downgradient of drinking water pipe networks even in the most forested watershed. Specific renderings of the drinking water distribution network are not shared by PWSA due to security reasons.

In addition to deep or shallow groundwater containing P originating from drinking water, other sources and processes can impact P flux to and within streams including immobilization in soils and sediment flux, respectively. P stored in streambed sediments is available as a P source, can diffuse to the water column and influence aquatic biota during summer baseflow conditions. The availability of sediment-stored P to the water column depends on a variety of interrelated factors, including 1) physical-chemical properties of the surrounding environment (oxygen content, temperature, pH, and porewater P concentration), 2) specific forms of P present (for example, Fe-bound P and Al-bound P), 3) hydrological and physical processes, and 4) biogeochemical spiraling such as absorption, adsorption and desorption [113–116]. In soils, sorption and desorption processes immobilize or mobilize P, respectively. P can be immobilized by adsorption whereby P molecules adhere to the surface of soil particles or by precipitation whereby P reacts with soil minerals to form calcium phosphate (alkaline soils) or iron/aluminum soils (acidic soils). In contrast, desorption, or the release of P from soil particles back into solution can result from a change in pH or organic matter decomposition. Additionally, weathering of P-rich minerals in geologic strata can also mobilize P impacting groundwater P and soil P concentrations [117,118]. Thus, while we observe significant increases in mean monthly TDP, TP, and TRP, in this study, the role of processes above on the observations reported here is uncertain.

Ecological significance of orthophosphate additions to drinking water

Although PO_4^{3-} additions to drinking water are an important practice for ensuring safe drinking water to the public, the impact of dosing extends to urban rivers where wastewater treatment plant effluent is discharged. While WWTPs remove a substantial portion of P during treatment processes, the resulting effluent discharged from WWTPs can still be a large source of P to aquatic ecosystems [26]. The Pittsburgh WWTP releases treated wastewater after completing primary and secondary treatments, and there are no tertiary procedures to remove P from the wastewater. This may be because Pennsylvania has no numeric water quality criteria for P; as such, the State does not have an effluent P limit for WWTP. In this study, we evaluated influent and effluent TP concentrations at the WWTP in the receiving area for PWSA customers, the Allegheny County Sanitary Authority (ALCOSAN). Over the two-year period encompassed by this study (January 2019 to December 2020), TP concentrations in ALCOSAN effluent discharged to the Ohio River had a significant increasing trend (Kendall's Tau = 0.54, $p < 0.001$) (S4a Fig). Compared to the pretreatment months (Jan-March 2019), TP concentrations in effluent for the same months in 2020 were significantly higher ($p < 0.05$) with median values increasing by 26% (S4c Fig). Although median values of influent TP increased by 12% for the equivalent time period, the difference was not significant,

and influent concentrations were generally highly variable over the study period (S4a Fig, S4b Fig). These findings suggest that the effects of drinking water PO_4^{3-} treatment extend beyond urban streams, placing additional pressure on WWTPs to manage elevated TP loads. Without effective removal, this excess P is likely to be discharged into urban rivers and other receiving waters, intensifying risks to ecosystem health, water quality, and regulatory compliance.

Conclusions and implications

This study provides compelling evidence of unintended ecological consequences stemming from public health interventions targeting Pb exposure. In our study system of Pittsburgh, PA, our findings indicate that high leakage rates from the drinking water distribution system, approximately 40–50% of the 70,000,000 gallons per day, resulted in concomitant changes in urban stream P and metal chemistry. The resulting cascade of environmental impacts on lotic ecosystems, including food webs, nutrient spiraling, nutrient limitation, primary productivity, and harmful algal bloom occurrence, has, to date, not been widely documented. This issue is especially critical considering the widespread distribution of Pb pipes across the U.S., particularly in the northeast, Midwest, and Great Lakes regions [26].

Overall, these findings underscore the need to (1) more explicitly explore connectivity between urban drinking water and urban stream systems, (2) develop best practices for WWTPs to manage the ecological implications of drinking water PO_4^{3-} additions, and (3) assess the relative influence of drinking water additions to eutrophication of urban rivers and downstream receiving waters. We recommend continued streamwater monitoring to determine whether elevated P and trace metal concentrations persist over time, as well as to determine how much P is deposited in sediments where it can later be released as legacy P. Expanding our understanding of these interlaced systems would improve the accuracy of predicting the persistence and extent of P sources, ultimately helping to better target efforts to reduce eutrophication on a global scale.

While it is imperative that humans are protected from Pb exposure, we also need to understand the full ecological implications of drinking water treatment for receiving water, be it groundwater, urban streams, or rivers. Thus, while PO_4^{3-} is an important option for protecting human health, future work should also evaluate the lowest possible PO_4^{3-} concentration that can be added to distribution systems to optimize the protection of both human and environmental health. This issue is especially critical considering a 2020 assessment that concluded that elevated P concentrations have resulted in 58% of U.S. river miles being classified as having poor water quality [119]. This persistent issue has contributed to the eutrophication of freshwater and marine ecosystems, a challenge that has spanned decades and continues to impact water quality nationwide.

Supporting information

S1 Table. Land-use cover, total area, and population density in five urban watersheds.
(DOCX)

S2 Table. Comparison of spring differences in streamwater pH and solute concentrations for the months of Mar-Apr-May 2019 (pre-treatment) and 2020 (post-treatment). Statistical significance was determined using Wilcoxen-Whitney test. Percent difference calculated using median values in 2019 and 2020.
(DOCX)

S3 Table. Responses ($p < 0.05$) of *Cylindrospermopsis* sp. biomass to various treatments (N as mg N/L and P as mg P/L) before PO_4^{3-} addition, two months, and twelve months after PO_4^{3-} addition for tap water and five urban streams. Only the significant responses are listed here.
(DOCX)

S4 Table. Responses ($p < 0.05$) of *Raphidocelis subcapitata* to various treatments (N as mg N/L and P as mg P/L) before PO_4^{3-} addition, two months, and twelve months after PO_4^{3-} addition for tap water and five urban streams. Only the significant responses are listed here.
(DOCX)

S5 Table. Nutrient limitation status of cyanobacteria (Cyano) (*Cylindrospermopsis* sp.) and green algae (Green) (*Raphidocelis subcapitata* to various treatments (No: No response to N and P additions; N + P: N and P co-limitation; N: N-limitation, and P: P-limitation) before PO_4^{3-} addition, two months after, and twelve months after PO_4^{3-} addition for tap water and five urban streams.
(DOCX)

S1 Fig. Boxplots indicate median and quartiles for (a) total dissolved phosphorus (TDP), (b) total phosphorus (TP), and (c) total reactive phosphorus (TRP) concentrations (μg P/L) before drinking water phosphate addition in five urban streams.
(TIF)

S2 Fig. Temporal trends in mean monthly phosphorus concentrations (μg P/L) after drinking water phosphate addition in five urban streams including (a) total dissolved phosphorus (TDP), (b) total reactive phosphorus (TRP), (c) total phosphorus (TP), and (d) the ratio of total nitrogen to total phosphorus (TN: TP). The increasing trend in concentrations of phosphorus across the study period, including TDP ($p < 0.0001$), TRP ($p < 0.05$), and TP ($p < 0.05$), were significant.
(TIF)

S3 Fig. Log-linear regressions between total dissolved phosphorus (TDP) concentration (μg P/L), and (a) manganese (Mn), (b) copper (Cu), (c) iron (Fe), and (d) lead (Pb) across five urban stream systems.
(TIF)

S4 Fig. Mean monthly concentrations of total phosphorus (μg P/L) are shown for (a) effluent (trend line) and influent (bar chart) from 2019 to 2020, and (b) boxplots indicating total phosphorus concentrations in influent during spring 2019 (before P addition) and spring 2020 (after P addition), and (c) effluent during spring 2019 (before P addition) and spring 2020. Data are sourced from the Allegheny County Sanitary Authority.
(TIF)

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