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Dual nitrogen and phosphorus reductions are needed for long-term mitigation of eutrophication and harmful cyanobacterial blooms in the hydrologically-variable San Francisco Bay Delta, CA

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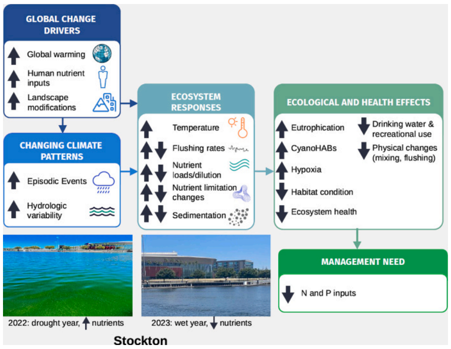
HIGHLIGHTS

- Hydrologic variability impacts nutrient dynamics in San Francisco Bay Delta.
- Record precipitation and prolonged snowmelt caused nutrient dilution at one site.
- At both sites, in wet and dry years, phosphorus (P) was replete for algal growth.
- When N was supplied, P enrichment further stimulated algal growth.
- Regardless of hydrologic extremes nutrient reductions are needed to control CHABs.

GRAPHICAL ABSTRACT



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ABSTRACT

Cyanobacterial harmful algal blooms (CyanoHABs) are a major concern for water quality, public health and viability of aquatic ecosystems. Increased inputs of nutrients, i.e., nitrogen (N) and phosphorus (P), are known to amplify the occurrence, severity, and duration of CyanoHABs. There is growing concern that CyanoHABs are proliferating along the freshwater to marine continuum, including throughout estuaries. We assessed the influence of nutrient enrichment on the abundance and composition of CyanoHABs and accompanying phytoplankton communities in the San Francisco Bay Delta (SFBD) estuarine ecosystem, a vital resource for California's water supply, fisheries, and recreation. In situ nutrient addition bioassays were conducted in June and September 2022, at the end of a record three-year drought period, and May and August 2023, an extremely high rainfall and discharge year. Water was collected from two locations in the SFBD recognized for having

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CyanoHAB issues, Discovery Bay (DB) and the Stockton Channel (STK). Both sites showed the highest proportion of cyanobacteria in the total phytoplankton community biomass during summer months, and this was particularly noticeable at STK. In June 2022, additions of N and N+P were both shown to increase overall phytoplankton biomass in DB and N+P specifically stimulated cyanobacteria. P alone was not stimulatory. In September 2022, NH_4 promoted the growth of cyanobacteria faster than NO_3, particularly in DB communities. A similar set of responses to N occurred in 2023 in DB, despite major differences in freshwater input between years. In 2022, nutrient additions had no significant stimulatory effects on STK phytoplankton communities, suggesting nutrients were replete throughout the bloom season. However, in 2023 N limitation became more evident in STK, likely due to a dilution effect from the very high freshwater discharge from a record snowpack and reservoir releases, ultimately changing the availability of inorganic N during the CyanoHAB growth period. The combined effect of high flow and nutrient dilution in 2023 was responsible for the reduced CyanoHAB potential. By examining these key differences between seasons in these hydrologically contrasting years, it appears that internal supplies of "legacy P" ensure P availability throughout the summer bloom season regardless of hydrologic variability, while N enrichment plays a key role in stimulating algal production and CyanoHABs under hydrologically variable conditions. Once N was added, P further stimulated biomass production in some cases, indicating potential N+P co-limitation. We conclude that under varying hydrologic conditions, long-term dual N and P input reductions are needed to control eutrophication and CyanoHAB outbreaks throughout the SFBD.

1. Introduction

Anthropogenic activities have accelerated eutrophication in aquatic systems worldwide due to increased nutrient (nitrogen-N and phosphorus-P) inputs from agricultural, urban and industrial expansion (Conley et al., 2009; Paerl et al., 2016; Wurtsbaugh et al., 2019). Previous research has shown strong links between increased N and P loads and the occurrence and expansion of cyanobacterial harmful algal blooms (CyanoHABs) along the freshwater to marine continuum (Smith and Schindler, 2009; Paerl et al., 2018; Wurtsbaugh et al., 2019). CyanoHABs pose a range of negative biogeochemical, ecological, and human-health impacts, including hypoxic events and fish kills, reduced growth of submerged aquatic vegetation, altered food webs and production of cyanotoxins (Brooks et al., 2015; Paerl and Otten, 2013; Plaas and Paerl, 2020). CyanoHABs are increasing in frequency and intensity in the San Francisco Bay Delta (SFBD), CA estuarine ecosystem and its riverine tributaries (i.e., the San Joaquin and Sacramento River systems) draining California's vast Central Valley (Lehman et al., 2013; Kudela et al., 2023; Preece et al., 2024a).

The SFBD is one of the largest estuarine systems in the United States (USGS, 2018). It provides water for municipal, agricultural, recreational, and industrial use while also providing key habitats for federally endangered and threatened fish species such as the Delta Smelt, Steelhead and Chinook Salmon (Bergamaschi et al., 2020; Preece et al., 2024a). The SFBD has been extensively modified by channelization, dams, diversions, levees, etc., which in conjunction with chronic freshwater withdrawal, has led to significant changes in the natural hydrological regime of the system (National Research Council, 2012). Furthermore, global and regional climatic changes have resulted in protracted droughts, increasing surface temperatures, and extreme precipitation events, coupled to record flooding, enhancing nutrient and sediment inputs (CA Dept of Water Resources, 2023). During droughts, these processes increase water residence times, raise temperatures, and coupled to excessive nutrient availability, allow for toxic CyanoHABs to outcompete more desirable eukaryotic algae (e.g. diatoms, chlorophytes) in the SFBD (Paerl and Otten, 2013; Lehman, 2022; Kudela et al., 2023).

Nitrogen (N) is the primary nutrient controlling primary production in most estuaries (Paerl and Piehler, 2008). The SFBD receives some of the highest N loads among estuaries worldwide, commonly exceeding U. S. Environmental Protection Agency recommendations (Cloern et al., 2020; Senn et al., 2020). Previous research has shown links between high concentrations of N in aquatic systems and increases in the occurrence and severity of CyanoHABs and the toxins they produce (Gobler et al., 2016; Chaffin et al., 2018, 2019); particularly, CyanoHAB species unable to fix atmospheric nitrogen (N_2), such as toxigenic *Microcystis* spp. (Krausfeldt et al., 2020; Paerl et al., 2019). Specific forms of N, especially NH_4, can influence the occurrence and persistence of CyanoHABs (Blomqvist et al., 1994; Newell et al., 2019; Wagner et al., 2021), as well as the production of their toxic secondary metabolites, e. g., microcystins and anatoxin-a (Barnard et al., 2021). There is debate however on the emergent roles of NO_3 and NH_4 on algal proliferation (CyanoHABs and more recently, the raphidophyte *Heterosigma akashiwo*) in the SFBD and its major tributaries, i.e., the Sacramento River and downstream San Francisco Bay (Dugdale et al., 2007; Parker et al., 2012; Strong et al., 2021; Cloern et al., 2020; Kudela et al., 2023).

The Sacramento and San Joaquin Rivers are the two primary routes for N to enter the SFBD. In the Sacramento River, 47 % of the delivered N load is from agricultural sources while 32 % is from wastewater treatment plant (WWTP) discharges, primarily in the form of ammonium (NH_4^+) (Saleh and Domagalski, 2021). In contrast, agriculture is the primary N source (62 %) to the San Joaquin River while point sources such as WWTPs contribute 16 % of N (Saleh and Domagalski, 2021). In summer months when inflows are lowest, and CyanoHABs are most problematic, with the two rivers each contributing about half the total nitrogen (TN) load, despite the San Joaquin River only contributing <20 % of the total inflows (Novick et al., 2015). It is generally agreed that there is a large and unused nutrient pool available in the SFBD that may present an increasing risk for CyanoHAB events (Preece et al., 2024b).

CyanoHABs have occurred in the upper region of the SFBD since the late 1990's, but in recent years, mixed assemblages of cyanotoxin producers, including *Aphanizomenon*, *Dolichospermum*, and *Microcystis* have proliferated (Lehman et al., 2021). In some areas of the SFBD, bloom-forming cyanobacteria can at times constitute up to 90 % of the primary producers, threatening water quality and integrity of planktonic food webs (National Research Council, 2012); with their cyanotoxins posing a threat to the health of communities living near, recreating in, or visiting the area (Plaas and Paerl, 2020; Preece et al., 2024c). Further, cyanotoxins are routinely found downstream in water and shellfish samples, presenting an unwelcome "export" to the marine waters of San Francisco Bay (Preece et al., 2017; Kudela et al., 2023). As such, there is an urgent need to better understand the environmental factors and nutrient inputs promoting CyanoHAB proliferation to identify effective mitigation steps. Specifically, we need to better understand the ecophysiological and nutrient dynamics that modulate growth of CyanoHABs and other phytoplankton taxa, given the range of hydrologic conditions characterizing the SFBD. Accordingly, here we investigated the role of eutrophication (specifically enrichment by NH_4, NO_3, phosphate [PO_4], and combinations) on algal growth potentials under the range of hydrologic condition and extremes impacting the SFBD. We compared phytoplankton community composition and group-specific growth responses to a range of individual and combined N and P enrichments under hydrologically varying conditions using in situ nutrient addition bioassays (Paerl et al., 2019; Barnard et al., 2021). Fortuitously, we were able to evaluate phytoplankton community responses to these key nutrients in successive extreme drought and wet years (2022−2023), in order to gauge how hydrological extremes impact nutrient-production dynamics in the SFBD.

2. Materials and methods

2.1. Study site and field collections

The convergence of the Sacramento and San Joaquin Rivers form the SFBD. The Sacramento River is the largest river in California and generates the majority of outflow into the SFBD. Water dynamics within the SFBD are governed by inflows from the watershed, tidal fluxes, and management actions that can impact flow paths and rates. The Central Valley Project (CVP) and State Water Project (SWP) export water via the California Aqueduct and Delta-Mendota Canal through a water conveyance system that supplies municipalities and agriculture to the south. These water exports account for approximately 15 % of the state's water supply and support 27 million Californians and 3 million acres of irrigated farmland (Sunding et al., 2023; Congressional Research Service, 2024). As pumps pull water south into the water conveyance system, it can cause reverse flows in parts of the central and southern SFBD, pulling water from the Sacramento River south through the SFBD instead of toward the San Francisco Bay and Pacific Ocean.

The SFBD has a typical Mediterranean climate, with dry, hot summers with most of the precipitation occurring during the wet season (October–March). Thus, inflows from the watershed peak in the spring and early summer from snowmelt and decline rapidly in the summer months. However, due to the intense management of the system, reservoir releases can be used to increase flows outside of the natural high flow period.

Water for bioassay experiments was collected from two designated field sites on June 6 and September 22, 2022, an extremely dry, low-flow year and May 19 and July 28, 2023, an extremely wet, high-flow year, reflecting early and mid-to-late summer bloom stages. One site was located in Discovery Bay (DB), a residential neighborhood located on a series of man-made canals that offshoot the Old River (37.905, −121.5878), and the second was located in the Stockton Channel, to the east of the Port of Stockton (STK) (37.954, −121.306) (Fig. 1). These sites were chosen due to recurring toxic CyanoHABs, as well as their different hydrologic properties, with DB being a semi-enclosed lagoonal system and STK being located at the terminus of a more riverine, although dead-end channel (Table 1).

2.2. Bioassay experiments

Water was collected at 0.5 m depth at each sampling site using a

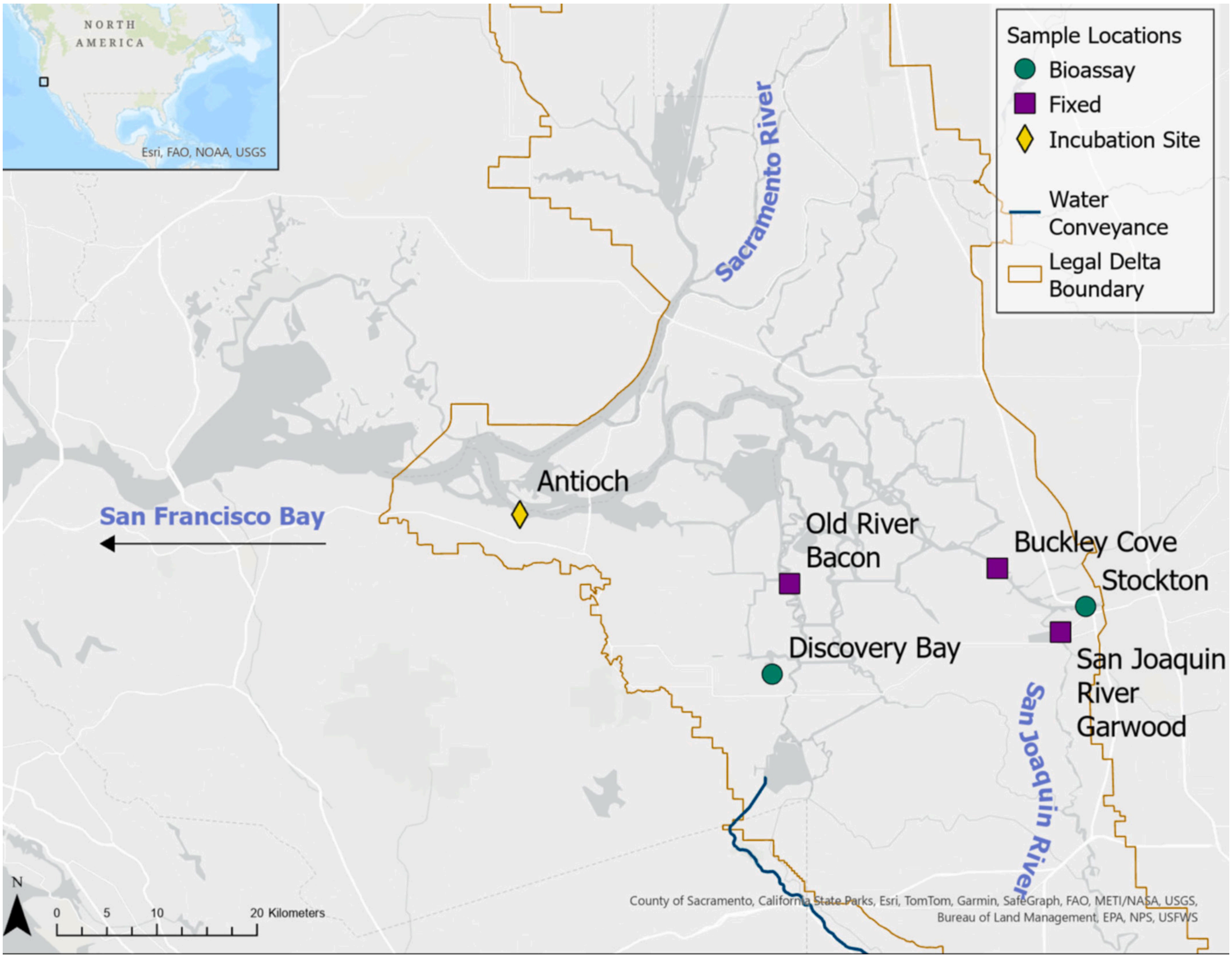


Fig. 1. Map of bioassay study sites at Discovery Bay (DB) and the Stockton Channel (STK), incubation study site in Antioch and fixed stations in Old River at Bacon Island (Old River Bacon), Buckley Cove, and the San Joaquin River at Garwood Bridge (San Joaquin River Garwood).

Table 1
Coordinates of study site locations in Discovery Bay, California.

Site	Abbreviation	GPS Coordinates
Discovery Bay Marina	DB	37.905139, −121.587857
5-Star Marina (on Stockton ship channel)	STK	37.953734, −121.305599
Jim's Holiday Harbor	Incubation Site	38.017707, −121.753230

submersible diaphragm pump, dispensed into pre-cleaned 20 L polyethylene carboys, and transported to the bioassay incubation site under natural light and temperature conditions at an accessible SFBD tributary near Antioch, CA (38.018, −121.753). Carboys were stored at ambient light and temperature, thoroughly mixed in a large plastic barrel and dispensed into pre-cleaned (0.1 N HCL, followed by sample water) Cubitainers (Hedwin Inc.) in 4 L aliquots for incubation. Cubitainers are 80 % PAR transparent, chemically inert polyethylene containers which are ideally suited for in situ incubations (Paerl et al., 2019; Barnard et al., 2021).

Nutrient concentrations for various treatments specific to each site were determined using previous monitoring data accessed through United States Geological Survey's online portal (Bergamaschi et al., 2020). Untreated control and the following five treatments were applied to DB samples in triplicate: 21.875 μM of NO_3 (KNO_3), 21.875 μM of NH_4 (NH_4Cl), 4.375 μM of P (KH_2PO_4), and a combined treatment of both combination of $NO_3 + PO_4$ and $NH_4 + PO_4$ (Fig. 2, S.I. Table 1). For STK, the same treatments were applied at 5× these concentrations per expected in the STK Channel: 87.5 μM of NO_3 (KNO_3), 87.5 μM of NH_4 (NH_4Cl), 17.5 μM of P (KH_2PO_4), and a combined treatment of both $NO_3 + PO_4$ and $NH_4 + PO_4$. In addition to experimental nutrient additions, 10 mg of dissolved inorganic carbon ($NaHCO_3$) and 43.75 μM of Silica (SiO_2) were added to each Cubitainer, including controls, to ensure these nutrients were not limiting during incubations. All treatments were consistent between the four experiments, apart from June 2022, for which NH_4 and $NH_4 + PO_4$ treatments were not conducted. A schematic of the bioassay setup is provided in Fig. 2. Cubitainers were then suspended in a floating corral off a dock at the Antioch site to incubate microcosm samples at ambient light and temperature conditions for six continuous days (Supplementary information (S.I.), Table 1). On experiment days 1 (T0), 2 (T1), 4 (T2), and 6 (T3), aliquots from each sample were collected and processed for biochemical analyses.

2.3. Laboratory analyses

To quantitatively determine the effects of nutrient enrichment on phytoplankton abundance and composition, samples were analyzed for chlorophyll *a* (Chl a) concentrations (overall phytoplankton biomass), and diagnostic phytoplankton pigments (specific algal group biomass; c.f. Pinckney et al., 2001), phytoplankton identification (microscopic confirmation), and soluble nutrient concentrations.

2.4. Phytoplankton pigment determinations

Chlorophylls and diagnostic phytoplankton pigment concentrations

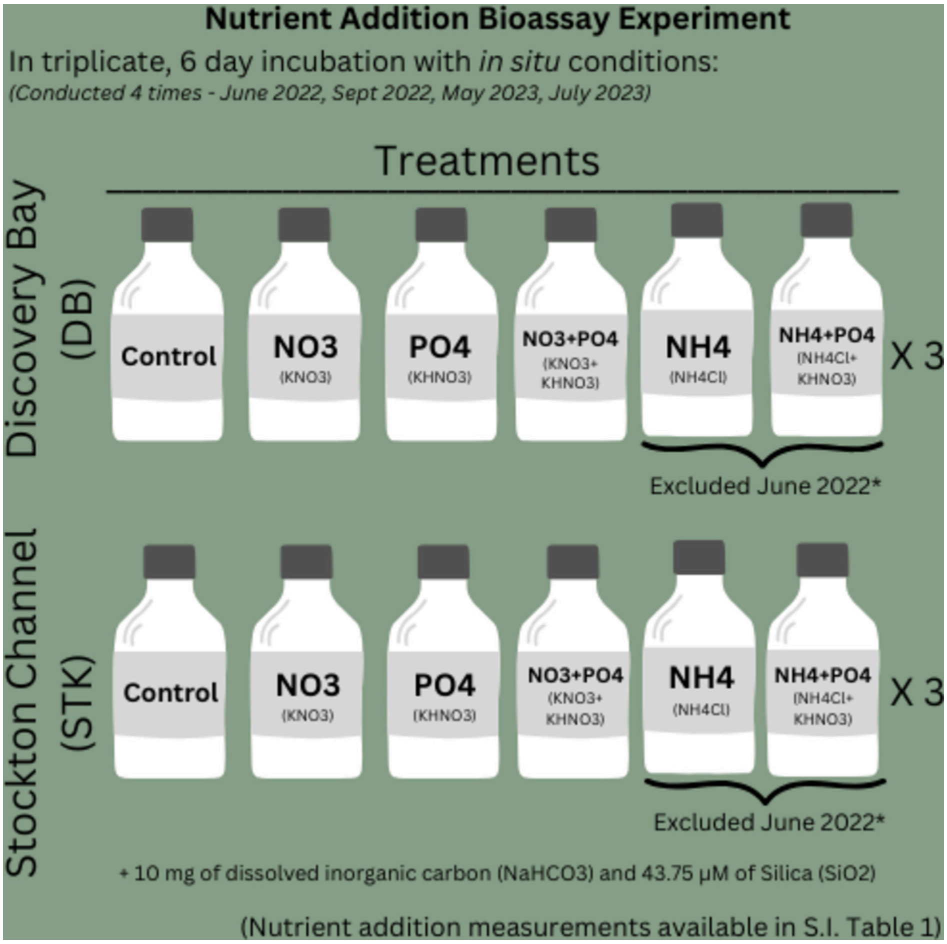


Fig. 2. Experimental set up for nutrient addition bioassay, depicting nutrients added to each treatment for both study site locations during all four experiments. Individual nutrient measurements can be found in S.I. Table 1.

were determined using high-performance liquid chromatography (HPLC) (Shimadzu model LC-20AB) equipped with an autoinjector (SIL-20AC) and photodiode array spectrophotometric detector (Shimadzu SPD-M20A). Pigment extraction was performed by submerging filters in MeOH, sonicating, and centrifugation. HPLC procedures are described by Pinckney et al. (2001). Major phytoplankton taxa and diagnostic pigments included chlorophytes or "green algae" (chlorophyll *b*, lutein, violaxanthin), cyanobacteria (zeaxanthin, myxoxanthophyll, echinenone, canthaxanthin), diatoms, raphidophytes (fucoxanthin, chlorophyll *c*, diadinoxanthin, diatoxanthin), dinoflagellates (peridinin), and cryptophytes (alloxanthin) (Jeffrey et al., 1999). Peaks were identified and quantified using *Shimadzu's LabSolutions Lite* software, and 19 distinct pigments were identified and quantified via a calibration curve generated from commercially available standards (DHI, Denmark). Pigment values listed as below detection were below the software threshold for peak detection or had spectra below a similarity of 0.9 compared to library spectra. Major algal groups were assigned via pigment biomarkers and confirmed via light microscopy for the identification of dominant species present in each treatment. Diagnostic photopigments were run through a matrix factorization program, ChemTax, to normalize pigment (carotenoids, Chl b) -specific biomass for each major phytoplankton group (cyanobacteria, chlorophytes, cryptophytes, diatoms, and dinoflagellates) as a fraction of total phytoplankton biomass, Chl a, as detailed in Mackey et al. (1996) and Schlüter et al. (2011). Members of major groups were confirmed manually using microscopic imaging.

2.5. Nutrient measurements

Soluble nutrient concentrations were determined utilizing a LACHAT QuikChem 8000 Flow Injection Analysis System following LACHAT Instruments' QuikChem Method 10-107-04-3-P for total dissolved nitrogen (TDN). The method detection limit for this analyzer is 93.34 μg/L for TDN. A SEAL QuAAtro39 Continuous Segmented Flow Analyzer (QuAAtro SEAL Analytical Inc.) was utilized following SEAL method guidelines in accordance with standard U.S. EPA methods to determine concentrations of phosphate (PO_4) (Method no. Q-037-05 Rev. 4), silica (SiO_2) (Method no. Q-005-04 Rev. 2), ammonium (NH_4) (Method no. Q-033-04 Rev. 8) and nitrate/nitrite (NO_3/NO_2) (Method no. MT3B Q-035-04 Rev. 10). Detection limits for this analyzer are as follows: 6.25 μ g/L NO_x, 1.49 μ g/L PO_4, 3.14 μ g/L NH_4, and 51.31 μ g/L SiO_2.

2.6. Data acquisition

Approximately 7 km from each of the designated field sites (i.e., DB and STK) are fixed water quality stations operated by the United States Geological Survey or the California Department of Water Resources. These stations collect a variety of continuous real-time data, including flow and electrical conductivity data. Directly adjacent to those fixed stations, the California Department of Water Resources collects monthly discrete samples that are analyzed for nutrients. Water quality station records were acquired through the California Water Data Library by querying the database for flow, and electrical conductivity data from water year 2022 (beginning October 1, 2021) through water year 2023, plus the first month of water year 2024 (ending October 31, 2023). Flow data were processed using a cosine lanczos filter to remove the tidal component of the time series and leaving only the net advection. Nutrient data were retrieved from the Environmental Data Initiative Data Portal (Battey and Perry, 2024).

2.7. Statistical analyses and data visualization

All data visualization and multivariate statistical analyses were accomplished through R version 7.2, using the 'vegan' package (Oksanen et al., 2022). Raw data and code can be accessed through a Github repository dedicated to this study (https://github.com/algaealex/CA-Bioassay-2022). For each bioassay, mean concentrations of pigments and nutrients were calculated along with standard error for unique treatments across time points. Differential algal growth between nutrient treatments was assessed by comparing chlorophyll *a*nd phytoplankton pigment concentrations with a one-way ANOVA test ($a = 0.05$). This was followed by post hoc multiple means comparisons (95 % simultaneous confidence intervals for specified linear combinations, by the Tukey method), performed on time point 3 (T3) data. Non-Multidimensional Scaling (NMDS) analysis with Bray-Curtis dissimilarity was conducted, as this analysis does not assume a normal multivariate distribution and does not place environmental constraints on the data (McCune and Grace, 2002). A Student's *t*-test was used to compare nutrient concentrations between years at the fixed stations.

3. Results

3.1. 2022 bioassay results

In 2022, early in the season prior to any nutrient addition, overall chlorophyll-a was higher at STK (43.78 ±1.75 μg/L) when compared to DB (25.88 ±6.5 μ g/L), but the relative abundance of cyanobacteria was higher in DB (Fig. 3). In DB, all additions of N, including NO_3, NH_4, NO_3+P, and NH_4+P, but not P alone, led to increases in total algal biomass (Chl a) and changes in community composition (Fig. 3). The relative abundance of cryptophytes and chlorophytes increased in both N and N+P treatment groups throughout the experiment. Cyanobacteria rapidly increased in their relative abundance in response to N-enrichment, as observed in the June experiment, and NO_3+P specifically led to the largest increase in cyanobacterial biomass. When comparing N treatments in September, NH_4 stimulated growth more readily when compared to NO_3 at equimolar concentrations (S.I. Table 2), as indicated by the drop in NH_4 concentrations and a sharp increase in all algal groups' biomass on T2 (Figs. 3 and 4); confirmed by a statistically significant increase between time points T2 and T3 ($p = 0.001$). Algal biomass under NO_3 enrichment showed a significant lower mean difference at T3 than in NH_4 or NH_4+P treatments. At the second time point (T2), NH_4 and NH_4+P were found to be significantly higher than NO_3, indicating NH_4 was preferred over NO_3 (S.I. Table 2).

Cyanobacteria, chlorophytes and cryptophytes dominated the community in DB in both June and September. This was also the case in June for STK, but in September, diatoms increased in relative biomass, leading to a more diverse phytoplankton community, still dominated by chlorophytes, cyanobacteria and cryptophytes, with little difference in relative proportions of phytoplankton groups between controls and any nutrient addition treatments throughout sampling times (Fig. 3).

For both June and September 2022, algal growth in STK site water exhibited no significant differences for any treatment group when compared to the control at each time point (Fig. 3, S.I. Table 2), suggesting that nutrients in the STK channel were replete prior to experimental manipulation, i.e., no nutrients were limiting to phytoplankton communities in the Stockton channel at any point during the 2022 bloom season (Fig. 3). However, there was a statistical difference in chlorophyll-a between NO_3 and NO_3+P treatments in June (Fig. 3, S.I Table 2), indicating greatest growth stimulation in response to combined N and P treatments ($p = 0.043$).

3.2. 2023 bioassay results

In DB, all N additions proved stimulatory, while P additions alone failed to stimulate phytoplankton biomass above controls. There was a slight but significant additional enhancement with N +P over N alone. This proved true for NH_4 +P and NO_3 +P, both in May and July bioassays. (Fig. 4, S.I. Table 2). Based on diagnostic pigment analyses, in the May DB bioassay, chlorophytes were the dominant biomass component, followed by diatoms and cyanobacteria. Interestingly, cyanobacteria only proved to be a significant fraction of total phytoplankton biomass in

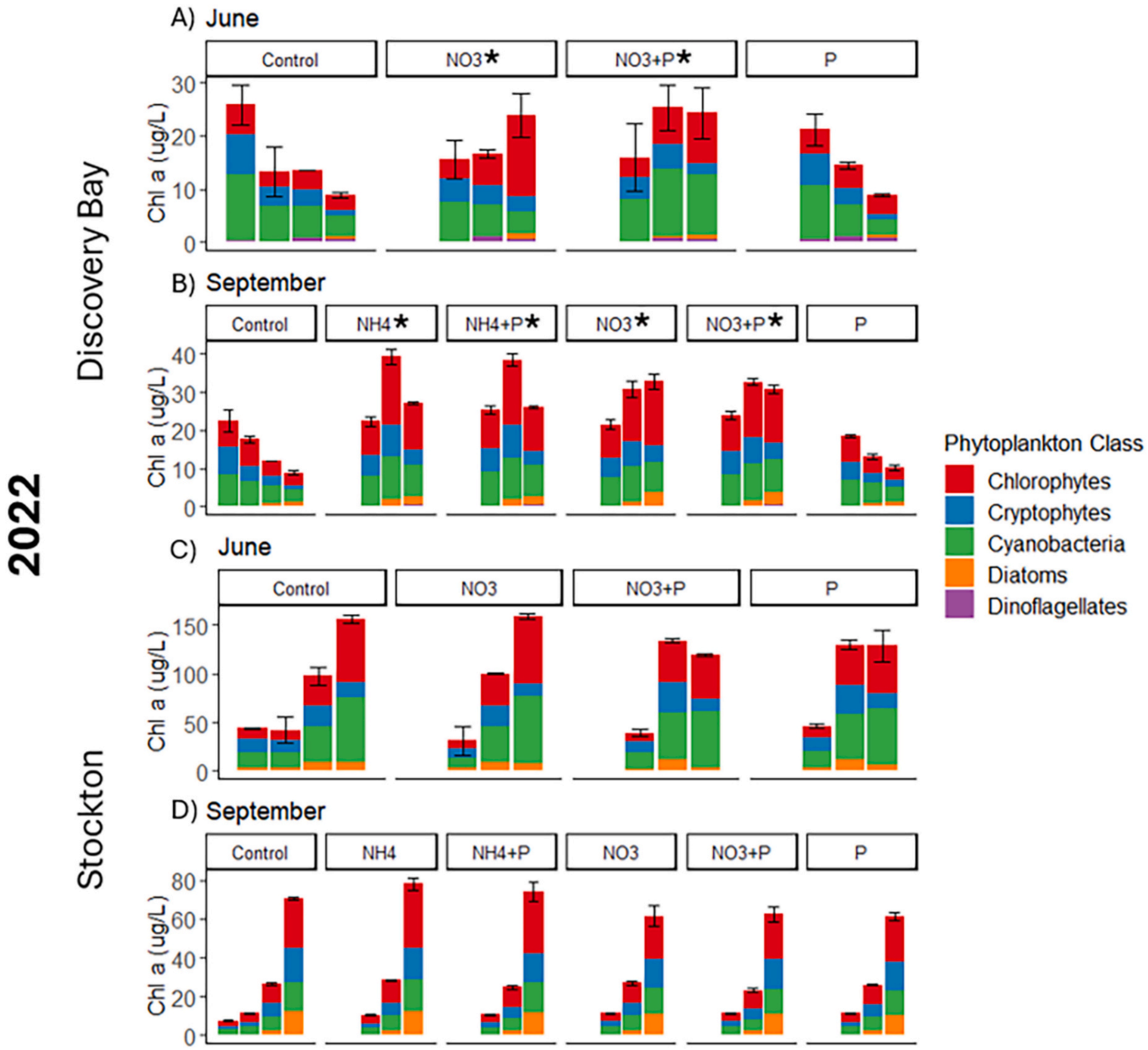


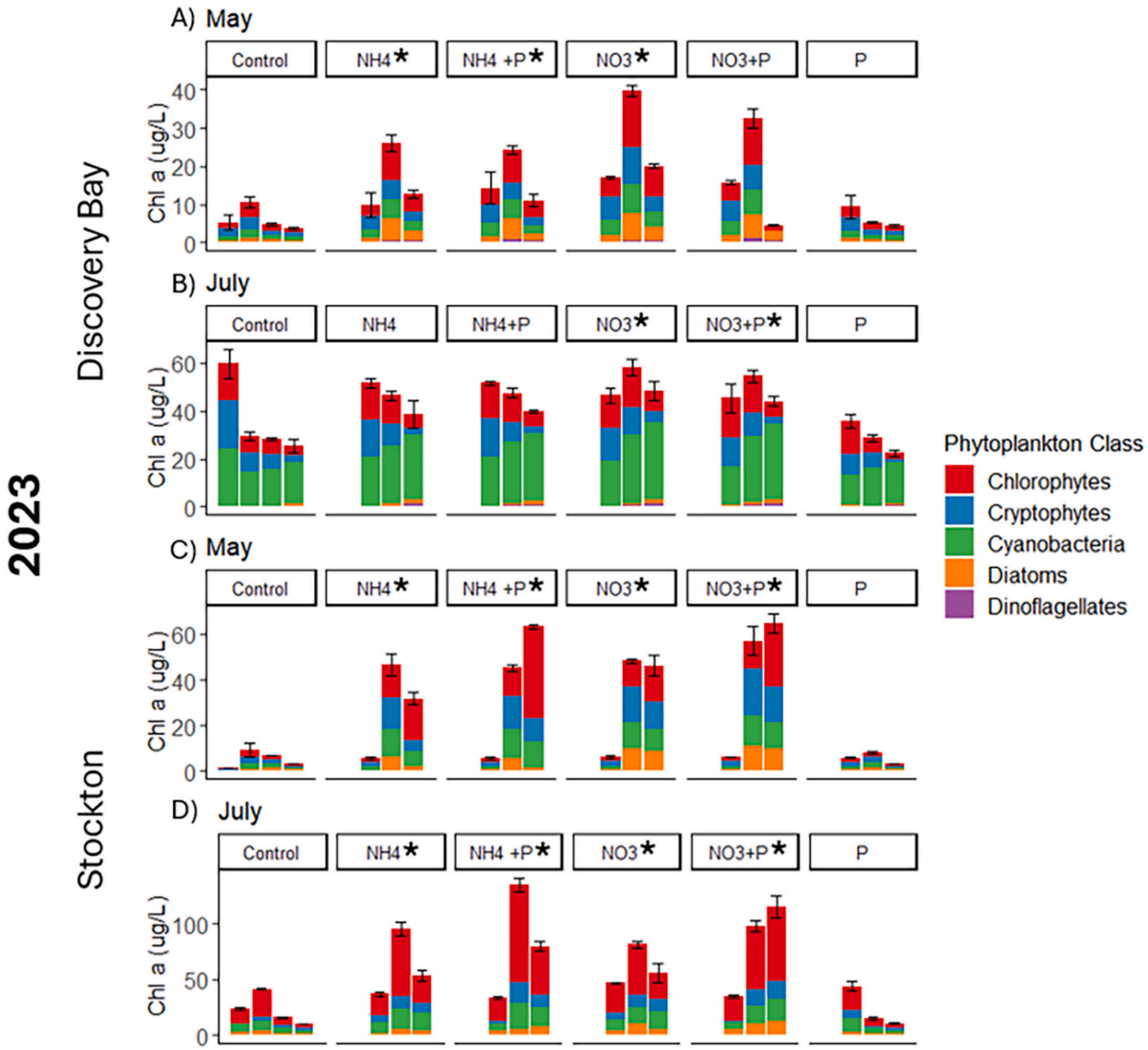
Fig. 3. Pigment concentrations (μg/L) representative of different algal groups were quantified by HPLC, normalized using Chemtax to determine the proportion of each pigment in the total Chl a. This was plotted across timepoints ranging from T0-T3 of the bioassay, and under the different treatments. A) and B) represent the Discovery Bay site in June and September 2022, respectively. C) and D) represent the Stockton site in June and September 2022, respectively. Asterisk indicates a significant difference (a = 0.05) in T3 chlorophyll *a* concentration compared to control group.

treatments that contained NH_4, as also seen in 2022 (Figs. 3, 4). This confirmed the preference that cyanobacteria have for NH_4 as an inorganic N source (Blomqvist et al., 1994; Newell et al., 2019). In the July DB bioassay, while diatoms remained a significant fraction of phytoplankton, chlorophytes and cyanobacteria increased in degree of dominance. Again, among the N sources, NH_4 proved to be most stimulatory, both alone and in combination with P.

For STK, the pattern of nutrient stimulation proved similar to DB, with all N containing treatments showing significant stimulation over controls, while P alone failed to stimulate over controls. Unlike the DB bioassays, NH_4 and NO_3 additions proved equally stimulatory. Also, in contrast to DB, STK N+P additions were not more stimulatory than N alone. With regard to STK phytoplankton community composition, in May chlorophytes, cyanobacteria and cryptophytes dominated, followed by diatoms. In July/August the composition remained similar, with a slight shift toward chlorophyte dominance, and diatoms assuming a lower percentage of the total biomass (Fig. 4).

A noticeable difference between 2022 and 2023 STK bioassay results was that while nutrient limitation was not evident in 2022, N limitation prevailed in both spring and summer bioassays in 2023 (Fig. 4). The higher flows in 2023 strongly affected STK nutrient concentrations, especially (nitrate + nitrite) and TDN (Fig. 5), which were much lower than similar periods in 2022. The lower nutrient concentrations reflect the dilution effect of high-volume rain and snowmelt driven freshwater discharge in 2023, compared to 2022, which was the last year in the driest three-year period on record (California Department of Water Resources, 2023) (Figs. 6, 7). This difference was most evident at the STK location because it is fed by the San Joaquin River, a major tributary of the SFBD which drains vast areas of the San Joaquin valley and snow melt from the Sierra Nevada mountains to the east. As a result, N limitation was highly significant there in 2023 (S.I. Table 2). The effect can also be seen in DB but is less profound, possibly because it is more influenced by the higher volumes of water that flow into the Delta from the Sacramento River (Fig. 6).

Net flows in Old River were much higher in 2023 relative to 2022 (when they were close to 0 cfs) (Fig. 6). However, for most of summer 2023 the flows were in reverse (i.e. flowing south) as high volumes of Sacramento River were pulled down through Old River directly to the pumps at the water export facilities in the southern Delta. In the San Joaquin River, which receives little, if any, impact from export pumps the flows were higher throughout the summer of 2023 relative to 2022 (Fig. 7). In 2023, while flows were elevated in both rivers, the electrical



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conductivity was suppressed to under 100 μS/cm. In contrast, during the 2022 drought year electrical conductivity was typically between 400 and 800 μS/cm as lower flows allowed salinity intrusion into the interior SFBD (Figs. 6, 7).

P concentrations remained consistent across all seasons and the two water years in Old River and the San Joaquin River (Figs. 6, 7). NH_4 also remained similar; however, NO_3 and TN were variable across seasons and years. In Old River, peak NO_3 and TN concentrations occurred during the first major runoff event, known as the "first flush." Although the first flush of 2023 carried more N than in 2022 by April of each year both NO_3 and TN were comparable (Fig. 6). In contrast, in the San Joaquin River near the STK site had significantly higher NO_3 ($p < 0.001$) and TN ($p < 0.001$) in the drought water year 2022 relative to the wet water year 2023 (Fig. 7). It was also notable that at this site, the first flush in 2022 carried higher N concentrations than in the wet water year 2023.

4. Discussion

The SFBD estuarine ecosystem is a hydrologically, biogeochemically and ecologically complex system influenced by a variety of anthropogenic stressors, including nutrient input from urban runoff, wastewater treatment facilities, agricultural runoff, and other sources (Dahm et al., 2016). It is important to consider how these anthropogenic influences impact phytoplankton community composition and abundance, and CyanoHABs, potentially leading to public and environmental health concerns in the rapidly expanding population centers it serves. The precise combined effects of nutrient input and environmental drivers (e.g., light limitation, temperature, inflow and residence time) underlying CyanoHAB expansion in the SFBD as explored herein remains the subject of ongoing research and modeling.

Our experiments indicate that equimolar additions of NO_3 and NH_4 both resulted in significant increases to total algal biomass at DB and STK, suggesting that SFBD phytoplankton communities effectively utilized either form of inorganic N and that P appeared to be replete. Algal growth at STK was slightly more stimulated by NO_3, while cyanobacterial communities in DB were more readily stimulated by NH_4 (Figs. 3, 4) suggesting that while there are differences in preference, both forms of N were readily bioavailable to phytoplankton communities in the SFBD. While we did not examine the role of dissolved and possibly particulate organic N, these N forms likely play additional roles in eutrophication and CyanoHAB dynamics, as shown in other estuarine

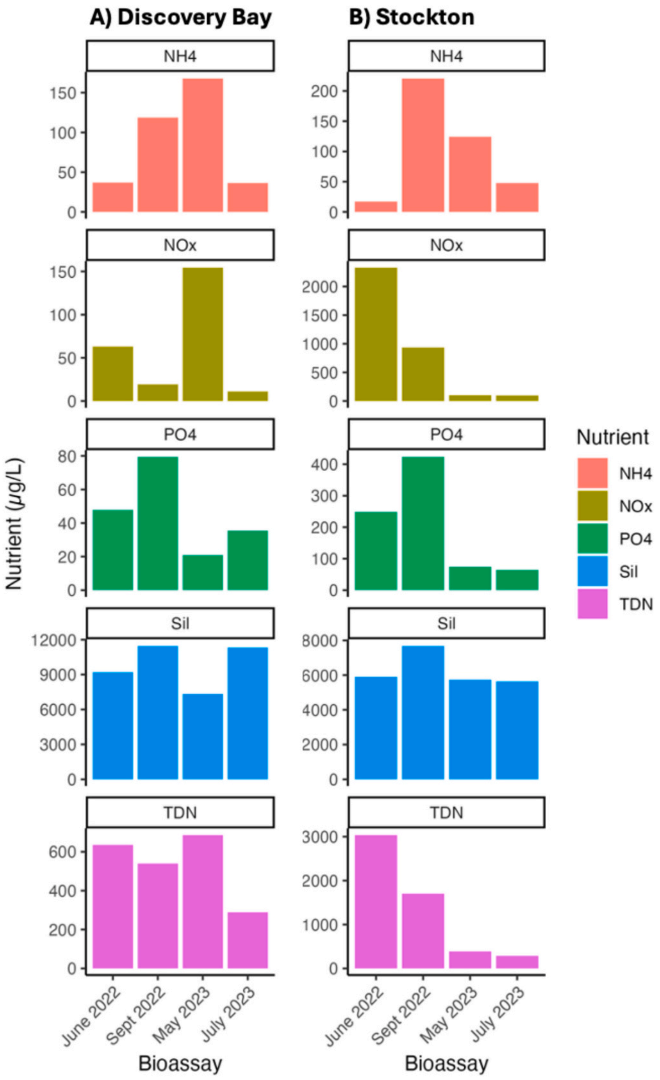

Fig. 5. Starting nutrient concentrations in 2022 and 2023 for A) Discovery Bay and B) Stockton Channel bioassays.

studies (Bronk et al., 2010; Altman and Paerl, 2012). While more research is necessary on phytoplankton specific uptake and growth rates in response to inorganic vs. organic N sources, we note that all inorganic forms of N, whether regenerated, recycled or externally supplied in the SFBD, should be included in management strategies to control N inputs. Given the rapid cycling of N in this system (Novick et al., 2015; Kendall et al., 2015; Lehman et al., 2015), N input controls should be based on total (dissolved and particulate, inorganic and organic) N forms.

While N appeared to be most limiting for STK and DB phytoplankton communities, treatments with N + P additions frequently resulted in maximum growth (Figs. 3, 4), suggesting N and P co-limitation of phytoplankton production in SFBD. Historically, nutrient limitation studies in freshwater ecosystems have linked eutrophication and bloom occurrences with inputs of soluble reactive P (Schindler et al., 2008). However, the “P only” nutrient-limitation paradigm has shifted over the past several decades, with more recent studies indicating that N + P co-limitation is common along the freshwater to marine continuum (Elser, 2007; Lewis et al., 2011; Paerl et al., 2016; Baer et al., 2023; McCullough et al., 2024; Rock and Collins, 2024).

Previous management approaches to dealing with the CyanoHAB problem focused on reducing phosphorus (P) inputs based on the fact that excessive P relative to N inputs (or low N:P ratios) were correlated with a tendency of receiving waters to be dominated by cyanobacterial biomass (Smith, 1983), with molar N:P ratios above ~15 discouraging CyanoHAB dominance (Smith, 1983; Smith and Schindler, 2009). In part, this predicted relationship was attributed to the fact that some common CyanoHAB genera (e.g., *Anabaena* (recently renamed *Dolichospermum*), *Aphanizomenon*) could fix atmospheric N_2 into biologically available NH_3, supporting the N requirements of bloom populations (Schindler et al., 2008). However, in the SFBD, non-N_2 fixing cyanobacteria (*Microcystis* spp.) tend to dominate and if concentrations of N or P are extremely high (i.e. above saturation levels), a ratio approach for reducing CyanoHABs is not likely to be effective or practical (Paerl and Otten, 2013). Furthermore, in shallow systems like the SFBD, sediment-water column exchange of nutrients is highly dynamic (Preece et al., 2024a). Hence, it is difficult to predict cyanobacterial dominance based on rather static N:P ratios (Paerl and Otten, 2013). Lastly, CyanoHABs are capable of storing either N or P as cyanophycins and polyphosphates respectively, further confounding a simple interpretation of nutrient limitation based on ambient N:P ratios.

In addition to nutrient loads, turbulence and mixing, residence time, irradiance, and temperature are the factors most strongly modulating growth and periodic proliferation of cyanobacteria in the SFBD (Lehman et al., 2021; Preece et al., 2024b). Drought years characterized by periods of low flow are correlated with a greater likelihood of high *Microcystis* biomass (Preece and Hartman, 2024). In contrast, during wet water years, high discharge can contribute high nutrient and sediment loads, but with more flushing in the system there are fewer bloom events (Lehman, 2022; Preece et al., 2024c). During the 2022–2023 experimental period, we experienced sequential low-flow drought and exceptionally wet scenarios (Figs. 6, 7). Even though freshwater discharge varied significantly between these years, we found that the patterns of nutrient limitation did not vary radically between these contrasting years, with N limitation most commonly encountered, and with N+P co-limitation being second most common. Interestingly, even at the nutrient enriched STK location, which showed nutrient replete conditions in low discharge 2022, N limitation was most common during high discharge conditions encountered in 2023.

During the drought year, the hydrograph was relatively flat as managers stored much of the water in upstream reservoirs during the rainy season and then during the summer months released water for peak demand and to maintain cool water temperatures for fish survival. In 2023, higher inflows occurred in winter and spring as a management response to flood risk. Higher than average flows continued into the summer months as prolonged snowmelt continued to fill upstream reservoirs and managers were able to release water to the water export facilities providing full supplies to their contractors for the first time since 2006. These high flows kept electrical conductivity lower than in drought years and diluted nutrient concentrations. This was especially evident near the STK site where NO_3 and TN concentrations appear to be closely related to inflows or lack thereof. In 2023, NO_3 and TN were significantly lower than in the drought year of 2022. In comparison, at the Old River site nutrients appear to have an opposite trend.

Channels throughout the SFBD with long residence times have been demonstrated to respond strongly to N inputs, resulting in higher cyanobacterial biomass, especially for non-N_2 fixing cyanobacterial species e.g., *Microcystis* spp. (Stumpner et al., 2020). Channels with shorter residence times exhibited rapid uptake of NH_4^+ and support phytoplankton communities composed of rapidly growing phytoplankton taxa, such as diatoms (Stumpner et al., 2020). During the bioassay periods, the high initial concentrations of cyanobacterial species in DB suggests longer residence times at this location, which is supported by stronger responses to N additions when compared to STK (Figs. 3, 4).

Our results also suggest that temperature plays a significant role in shaping phytoplankton community composition, with cyanobacteria and chlorophytes showing dominance during warm summer periods. This underscores the important role that water column warming plays in enhancing the competitive advantage that CyanoHABs have in a warming climate (Paerl and Huisman, 2008, 2009), which the SFBD

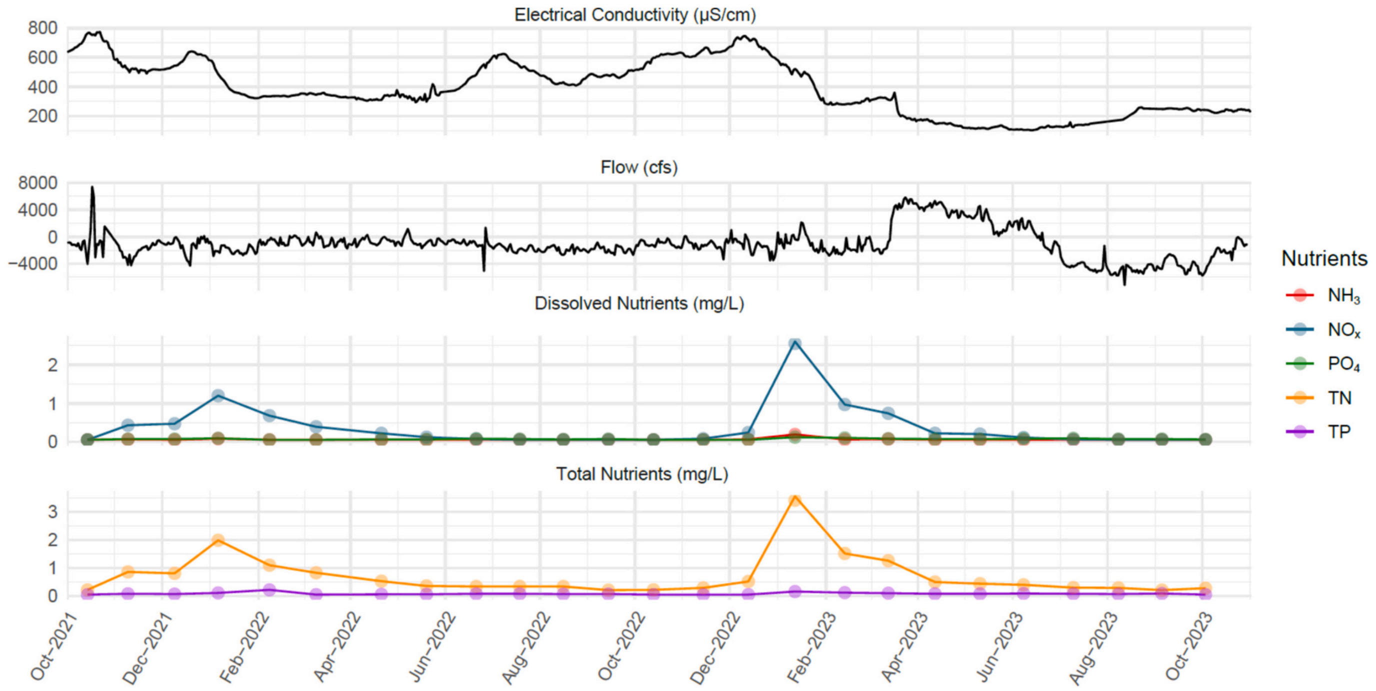

Fig. 6. Continuous (in 15-min increments) electrical conductivity and flow data from the United States Geological fixed station located in Old River near Bacon Island. Discrete nutrient data collected by the California Department of Water Resources in Old River near Bacon Island (Station D28). Data from the California Water Data Library and Environmental Data Initiative Data Portal. A bold "B" indicates when each of the four bioassays were conducted.

Fig. 7. Continuous (in 15-min increments) electrical conductivity at the California Department of Water Resources fixed station in the San Joaquin River near Garwood Bridge. Continuous (in 15-min increments) flow data at the California Department of Water Resources fixed station in the San Joaquin River near Buckley Cove. Discrete nutrient data collected by the California Department of Water Resources in in the San Joaquin River near Buckley Cove (Station P8). Data from the California Water Data Library and Environmental Data Initiative Data Portal. A bold "B" indicates when each of the four bioassays were conducted.

exhibits (Dettinger et al., 2016). However, since temperature is not manageable and flow manipulation is limited in this complex system, we are left with strategic external nutrient input reductions to mitigate the CyanoHABs and their cyanotoxin production in this system (Preece et al., 2024a).

Dual nutrient reduction strategies are especially important along estuarine gradients (Paerl and Huisman, 2009; Paerl et al., 2016, 2018) including the SFBD, where phytoplankton communities, whose growth rates are maximized under different salinity and nutrient regimes, vary progressively downstream (Preece et al., 2024b). Reducing P inputs may mitigate eutrophication in upstream riverine segments, but this will remove the “filter” for reducing N in the more N-limited downstream deltaic waters (Paerl, 2009). Thus, without a co-reduction of N, the blooms are likely to shift in their composition and location along an estuarine gradient, still negatively impacting water quality (Paerl, 2009; Paerl et al., 2011). Accordingly, both N and P inputs should receive priority over single nutrient reductions for the SFBD. Over the past few years, several Wastewater Treatment Plants (WWTPs) have undertaken costly, and major, process upgrades to reduce their loading of dissolved inorganic nitrogen (DIN) to the SFBD. This includes the 2021 upgrade to one of the largest WWTP in the area, located on the lower Sacramento River. Prior to the upgrade the WWTP could provide up to 90 % of the DIN, mostly in the form of NH_4^+, to the lower Sacramento River during summer months (Mussen et al., 2024). The upgrade converted NH_4^+ to NO_3 and removed >50 % of the NO_3 from the WWTP’s effluent. Although outcomes of these N load reductions have yet to be fully understood, the reduction of point sources is a step in the right direction (Glibert et al., 2022). As sources of nitrogen from WWTP’s diminish the focus on nutrient control must shift to upstream watershed sources such as agriculture (Saleh and Domagalski, 2021).

5. Conclusion

We conducted summertime in situ nutrient addition bioassays during contrasting hydrologic years in the SFBD of California. Findings indicated that dominant inorganic forms of N, NH_4 and NO_3, were primarily limiting to phytoplankton communities in both 2022 and 2023; however, dual N and P reductions most readily stimulated and sustained growth over the experimental periods. This suggests that both N and P input reductions are needed to counter eutrophication and mitigate the development and proliferation of CyanoHABs in the SFBD. Accordingly, we recommend that managers and policymakers focus on continuing to reduce inputs of total N from the SFBD watershed, accounting for all inorganic and organic forms. Parallel reductions to total P should also be practiced given that internal cycling between inorganic (PO_4) and organic forms of P is similarly dynamic. Furthermore, P tends to build up as “legacy P” over years to decades. Both total N and P reductions should be implemented on a year-round basis, since water residence times and internal storage of these nutrients can vary substantially during drought/flood cycle impacting the SFBD. Conducting further research into the seasonal threshold levels at which nutrients need to be reduced to mitigate these blooms will aid in management and policy decisions regarding the SFBD and its watershed.

CRediT authorship contribution statement

Hans W. Paerl: Writing – original draft, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Haley E. Plaas:** Writing – original draft, Investigation, Conceptualization. **Leah M. Nelson:** Writing – review & editing, Methodology, Investigation. **Alexandrea Sabo Korbobo:** Writing – review & editing, Methodology, Data curation. **Jack H. Cheshire:** Writing – review & editing, Project administration, Methodology, Formal analysis, Data curation. **Lindsay Yue:** Writing – review & editing, Methodology, Formal analysis, Conceptualization. **Ellen P. Preece:** Writing – review & editing, Methodology, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Hans Paerl reports financial support was provided by National Science Foundation, National Institutes of Health (NIEH). None if there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.177499.

Data availability

Data will be made available on request.

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