Spatial Biomass Production And Seasonal Nutrient Limitation Transitions In A Tributary of The Three Gorges Reservoir, China

Amechi Sampson Nwankwegu (✉ smetchie@yahoo.com)
Hohai University  https://orcid.org/0000-0003-3561-0514

Yiping Li
Hohai University

Lei Zhang
Southwest University

Yanan Huang
Hohai University

Deti Xie
Southwest University

Eyram Norgbey
Hohai University

Hans W. Paerl
The University of North Carolina at Chapel Hill

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Abstract

The freshwater ecosystem characteristics in terms of nutrient inventory across seasons, spatial variations of chl-a biomass, and the phytoplankton community structure are prudent ecological assessment indices for a bloom management protocol. We evaluated the spatial and seasonal chl-a distribution under different nutrient conditions and phytoplankton community structure in a eutrophic Three Gorges reservoir tributary China. Result showed significant variations in biomass production with the mainstream reaches severely affected. The nutrient addition bioassay demonstrated significant stimulations on growth in both autumn and summer. The nutrient limitation pattern shifted from P in autumn and spring to N limitation during summer. Combined additions of trace metals with N, P, and Si in autumn and Fe alone enrichment in summer and spring showed maximum productivity. The phytoplankton community structure demonstrated strong sensitivities to seasonal variabilities with regime shift from Cyanophyta, dominated by the toxic and hypoxia generating, *Microcystis spp* in both autumn and summer, the Cryptophyta dominated by the *Chroomonas acuta* in spring to the Bacillariophyta dominated by the genera, *Cyclotella* in winter. This reflected the ability of the Bacillariophyta to thrive under a low-temperature condition. Combined N&P led to significant growth stimulation in summer while P alone controlled the bulk of the growth in autumn. The study points to the need for extending mitigation steps to the mainstream towards achieving lasting bloom management solution in the impacted tributary.

Introduction

Eutrophication, which adversely affects both the chemical and ecological status of aquatic ecosystems worldwide has remained unabated despite several mitigation approaches (Nwankwegu et al., 2019). Aside from the physicochemical and chlorophyll-a (chl-a) concentration, other water quality indices for bloom assessment are till date controversial (Sòria-perpinyà et al., 2019). While the former focuses on the quantification of the nutrient inventory, nitrogen (N), phosphorus (P), as well as some physical parameters including; pH, dissolved oxygen, and (DO), the later provides information on the relative phytoplankton community biomass and assesses the eutrophic status of aquatic systems. The biomass production as chl-a represents a more direct measure of the extent of primary productivity and bloom severity in the eutrophic freshwaters. The magnitude of biomass yield during an intense bloom can also provide a presumptive ecological assessment on the specific genera/species dominating a bloom (Nwankwegu et al., 2019). The physiochemical index merely assesses the implications of the increasing anthropogenic activities including N& P inputs loading, as well as some pH and DO-altering chemicals. This index, unlike the chl-a concentration certainly does not address concerns relating to the phytoplankton community structure. The chl-a estimation is thus most common index for the trophic state categorization, impact indication, and water quality management (Zou et al., 2020; Sòria-perpinyà et al., 2019; Carneiro et al., 2014). Further, based on the European Water Framework Directive, chlorophyll-a and cyanobacteria have been adopted in the ecological classification of freshwaters e.g., lakes
(Søndergaard et al., 2011) perplexed by the multiple climatic, anthropogenic, and environmental stressors.

In the eutrophic Xiangxi Bay (XXB), a Yangtze River tributary in Hubei Province, China, bearer of the world’s largest impoundment project, the Three Gorges Dam (Gao et al., 2018), extended monitoring campaigns in terms of the spatial biomass production and the seasonal nutrient limitation transitions have not been considered in the past 15 years. We are also not aware of any recent study that has considered the full report of the XXB seasonal pattern of nutrient limitation shift, microelements stimulation on growth, and spatial biomass yields, particularly during the productive period. Several previous studies in the tributary have evaluated the spatial analysis of biomass approximately 15 years ago e.g. spring bloom and nutrient limitation (Fang et al., 2013; Xu et al., 2009; Ye and Cai, 2009; Cai and Hu, 2006; Ye et al., 2006; Ye et al., 2007), the effect of Three Gorges Reservoir (TGR) impoundment and temporal coherence for water clarity (Bi et al., 2010; Xu et al., 2010), the longitudinal difference in phytoplankton community structure (Wang et al., 2011a, 2011b, 2011c, 2010d; Zeng et al., 2006), vertical mixing regimes and current characteristics (Liu et al., 2012; Yang et al., 2018), the sediment P-release (Fu et al., 2006; Zhijing et al., 2012), and XXB critical nutrient isotopes (Yang et al., 2015). However, these studies did not consider the trace metals interactions with the representative phytoplankton groups.

Considering the constantly changing climate and increasing anthropogenic actions (Nwankwegu et al., 2019) from urban discharge, agro-fertilizers, and the mining activities in the XXB catchments, the eutrophication impacts, trace metal pollution, and the seasonal pattern of the phytoplankton dominance in the tributary are likely to have changed over time. The few recent studies which involved microelements, nutrient limitation, phytoplankton community structure and water quality trends (Nwankwegu et al., 2020a; Huang et al., 2020; Li et al., 2019), are potentially limited by time as these studies characterized the ecosystem functions based on single-seasonal outcome and conclusions drawn from a point monitoring evidence which may not essentially apply to the other points across the tributary to the mainstream. Again, a recent study which evaluated the spatial distribution of microelement in the entire bay only considered iron (Fe) alone (Nwankwegu et al., 2020b). Spatial biomass accumulation during productive periods, the seasonal nutrient limitation, as well as growth stimulation by microelements are particularly imperative to infer the contemporary eutrophication impacts in the vulnerable ecosystem.

In the present study, we, therefore, studied the spatial variations in the distribution of chl-a biomass along the XXB major representative reaches to the mainstream during the intense summer bloom. We also evaluated the seasonal dominance in the phytoplankton taxonomic groups across seasons (autumn, winter, spring and summer), and ascertained the seasonal variations in the critical nutrient pool controlling phytoplankton growth. Further, we constituted nutrient addition bioassays involving the principal nutrients (N&P), as well as the micro-nutrients; silicon (Si), iron (Fe), zinc (Zn), manganese (Mn), and copper (Cu) to assess their responses on growth promotions while estimating the synergy among the different nutrient combinations. We hypothesized a significant difference in biomass production between the mainstream and the other points within Xiangxi Bay. The present study would provide useful insights into effectively understanding the aquatic ecosystem functioning while complementing the current
mitigation design in the management of the unabated bloom in Xiangxi tributary and related freshwaters under similar anthropogenic stressors.

Materials And Methods

2.1. Study area

Xiangxi Bay (XXB) is located in Xiakou Town, Yichang City, Hubei Province, China within the latitude 31°04′ – 31°34N and longitude 110°25′ – 111°06E. It has the annual watershed area of 3099 km², 47.4 m³/s annual flow, and about 94 km in length. It is the largest tributary in the downstream Yangtze River potentially under the anthropogenic stressors induced by the hydraulic structure, the Three Gorges Dam (TGD). It empties into the mainstream of the Three Gorges Reservoir (TGR) in Xiangxi Town and flows through Xingshan and Zigui Counties, north to south (Yang et al., 2015). It is characterized by subtropical continental monsoon climate (Nwankwegu et al., 2020a). The XXB originated from the accumulated effect of the operations of the Three Gorges Dam (TGD) following the initial impoundment in 2003 (Ji et al., 2017; Jiang et al., 2018; Zhu et al., 2013). The hydrodynamic and hydrologic influences created a bay essentially recharged by the backwater intrusion extending to 40 km upon reservoir closure for water retention during low flow seasons (Jiang et al., 2018). The bay shares typical attributes of stable systems e.g., lakes including long residence time (131 d), thermal stratification, eutrophication, and blooms (Nwankwegu et al., 2020a; Xu et al., 2011) affecting the spatial heterogeneity along the tributary (Soares et al., 2012). The bay is heavily impacted by multiple anthropogenic stressors including; the direct discharge of untreated domestic sewage, agricultural runoffs due to the commercial row-crop agriculture (Nwankwegu et al., 202b) dominated by leguminous crops and potentially increasing the ecosystem N budget. The in-situ laundry services by the local people of Xingshan and Zigui counties also accounts for the enormous phosphorus (P) input into the system. The annual phosphorus load has been estimated to 29.78 tons (Nwankwegu et al., 2020a) and 61–40% of TN and TP inputs have been attributed to the non-point sources from the upper XXB tributary (Yang et al., 2015). The mainstream nutrient structure has been described far beyond the internationally recognized eutrophication threshold (Zhou et al., 2011; Nwankwegu et al., 2020a). There are also several industrial localization within the XXB bank, typical examples are the Shu Kongping chemical and mining industrial in Gufu and Xiakou towns, the end of backwater zone, which are the potential sources of anthropogenic nutrients, especially phosphorus leaching from waste rocks into the XXB watershed (Jiang et al., 2016). The nutrient characteristics without a proportionate tidal inundations make the bay a typical nutrient-saturated system (Dai et al., 2010; Zhijing et al., 2012) and the harmful cyanobacterial blooms have remained unabated in recent years despite several mitigation attempts.

2.2. Nutrient addition bioassay experiment

Sampling was conducted over one year from August 2018 to August 2019 aimed at covering the four seasons; autumn, winter, spring and summer. The sampling points for the spatial chl-a distribution during the intense summer bloom were; the downstream reach (XX01), the middle reach of XXB (XX06)
otherwise known as the XXB confluence, which is 18.8 km from the TGR, and the mainstream TGR (XX09), 32 km from XXB. The collected samples were returned to the platform at XX06 for incubation (see Fig.S1). The location XX06 was used in other seasons while the bloom intensity was low. The navigation of the sparsely distributed sampling points was made possible with the use of a steamer vessel belonging to China's Three Gorges University, Yichang, Hubei province. Nutrient addition bioassay experiments were deployed in XXB during autumn (20th August – 15th September 2018), winter (20th December 2018–9th January 2019), spring (5th April- April 2019), and summer (19 July- 1st August 2019). Subsurface (approximately 0.2 m) water samples were collected into a cleaned plastic container (280 L) which was 0.01 N HCl-sterilized and XXB water-rinsed. The chlorophyll-a (chl-a) biomass surface scum was scooped off during water samples collection to obtain a moderate initial biomass density of approximately 10 µgL⁻¹ (Xu et al., 2015) during summer and autumn when primary production was most severe with the characteristic large surface cyanobacterial "mats". Water samples pre-screening for zooplankton removal was not considered. This is because the modification significantly alters the baseline phytoplankton assemblages while increasing the bias associated with the extrapolation of the bioassay outcomes to the natural community structure of phytoplankton (Li et al., 2020; Domingues et al., 2016). In natural eutrophic freshwater, the complex interactions often do not stop the harmful algal bloom expansion. So, removal of the non-plankton diversity by filtration can produce results which cannot accurately represent the natural ecosystem characteristics. Further, particulate nutrients and some planktonic algae may be held within the bloom biofilm and are removed during filtration (Li et al., 2020). This potentially creates artificial phytoplankton community structure and nutrient limitation pattern that could differ significantly with the ecosystem actuality. The pooled water sample was continuously stirred for even distribution of nutrients, particles, and phytoplankton species while dispensing 4 L aliquot into acid (0.01 HCl) and then XXB water-washed 5-L polyethylene Cubitainers (Paerl et al., 2011; Xu et al., 2013; Xu et al., 2015). The Cubitainers are unbreakable, foldable, chemically inert, transparent and permit ≥ 80% PAR transmittance (Xu et al., 2015; Paerl et al., 2011; Paerl and Bowles, 1987) thus are remarkably useful in bioassay experiments. The microcosms consisted of the following nutrient enrichment; nitrogen (N), phosphorus (P), (NP), silicon (Si), (NPSi), and trace metals hereafter referred to as Ms, which composed of iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu). These were added in a single saturating pulse as follows; N was supplied in two forms; KNO₃ for nitrate (NO₃) and NH₄Cl for ammonium (NH₄), P as K₂HPO₄, Si as K₂SiO₃, metals (Fe, Zn, Cu, and Mn) as FeCl₃, ZnSO₄, CuSO₄, and MnSO₄, respectively. To evaluate the co-stimulatory role of Fe with the different N forms and P; extra bioassay microcosms were set-up as (N03 + Fe), (NH₄ + Fe), and (P + Fe). Each group of the bioassay involved the control which did not receive any nutrient enrichment. The final concentrations of all nutrients were 1.0 mgNL⁻¹ for N (N03 and NH₄), 0.1 mgPL⁻¹ for P, 6 mgSiL⁻¹ for Si, and 0.1 mgL⁻¹ each for the trace metals. These concentrations were based on the nutrient characteristics in XXB reported in our previous studies (Li et al., 2020; Huang et al., 2020; Nwankwegu et al., 2020a). The concentrations were intended to be higher than the in-situ concentrations to alleviate any potential scenario of nutrient limitation that could mask the effect of other factors essential for growth (Li et al., 2020; Domingues et al., 2016; Domingues et al., 2017). The incidence of carbon limitation due to starvation stress was
addressed by supplying external carbon source as 10 mgL$^{-1}$ KHCO$_3$ (Nwankwegu et al., 2020a; Nwankwegu et al., 2020b; Xu et al., 2015) across the microcosms including the control at the beginning of the study. Following nutrient enrichments, Cubitainers were incubated in-situ while placing them over floating frames allowing for natural light, temperature and wave action (Paerl et al., 2011; Xu et al., 2015). Considering that bioassay is used for the immediate assessment of phytoplankton-nutrient interactions under a controlled condition and short incubation period (Nwankwegu et al., 2020a; Xu et al., 2013), incubation lasted for 4 d in each season. This helped to reduce the chances of toxic metabolite production while minimizing the potential “bottle effects” (Domingue et al., 2016; Xu et al., 2015). A layer of neutral density screening (e.g., mosquito net) placed over the incubating frames was used to prevent photo-inhibition during the incubation (Paerl et al., 2011). This helped reduced the incident radiation by 30% (Xu et al., 2013) since increased ultraviolet radiation (UVR) can elicit a negative effect on phytoplankton (Nwankwegu et al., 2020a; Domingues et al., 2016) and largely affects optimal chl-a pigmentation. The Cubitainers were sampled for nutrients and chl-a concentrations at 1–2 d interval.

### 2.3. Water sample analysis

**In-situ** measurement of pH, temperature, dissolved oxygen (DO), and electrical conductivity were performed using multi-sensor Yellow Spring Instrument ((YSI Quatro, 18G100594, USA)) automatic calibration aided. Total nitrogen (TN), total phosphorus (TP) and dissolved nutrients TDN, and TDP were analyzed by combined persulfate digestion and further followed by spectrophotometric analysis for nitrate-nitrogen (NO$_3^-$-N). Ammonium nitrogen (NH$_4^+$-N) was analyzed using the indophenol blue method (Huang et al., 2020). Biological parameters which included identification of phytoplankton genera and chlorophyll a (Chl-a) also followed. Phytoplankton biomass estimation was quantified by chlorophyll (chl-a) concentration analysis. Briefly, 300 mL of the water sample was filtered onto 25 mm GF/F filters, gently folded and blotted dry, placed in foil wrappers and then kept frozen until used. After extraction in 90% hot acetone, chl-a concentration was quantified spectrophotometrically (Paerl et al., 2015). Phytoplankton samples were fixed with Lugol's iodine solution (2% final concentration) and settled for 48 h and identified (Li et al., 2020; Huang et al., 2020). Algal cell density was microscopically measured using a Sedgwick - Rafter counting chamber at magnifications of 320–400x (Nwankwegu et al., 2020b; Xu et al., 2013). Species of phytoplankton were identified and grouped into their corresponding taxa and seasons.

### 2.4. Statistical analysis

Each set of data in the experiments was collected in three replicates and the analytical result was the mean of three measurements. The standard deviations (error bars) and statistical significance (5 % level of significance) were analyzed with origin software. Differences in the growth responses (chl-a) between various treatments were analyzed by one-way ANOVA. The Post Hoc Multiple comparisons of treatment means across seasons were performed by Tukey’s least significant difference procedure. The classical growth rate (µ) equation mathematically expressed as;
\[ \mu = \frac{\ln(X_2-X_1)}{(T_2-T_1)} \]

was adopted in estimating the growth rates based on chl-a influenced by the nutrient enrichments across the treatments and sampling points. Where \( X_1 \) is the concentration of chl-a at \((T_1)\), and \( X_2 \) is the concentration of chl-a at the time \((T_2)\) (Huag et al., 2020; Nwankwegu et al., 2020a; b).

**Results And Discussion**

### 3.1 Spatial chl-a distribution and effect of nutrient additions on growth

The different locations of XXB during summer showed strong spatial variabilities in the chl-a distributions as demonstrated by the control across the three points (Fig. 1); XX01 (19.41 ± 3.29 µgL\(^{-1}\)), XX06 (38.98 ± 4.04 µgL\(^{-1}\)), and XX09 (183.02 ± 28.31 µgL\(^{-1}\)). For XX01 and XX06 in day 2 (Fig. 1a), all nutrients addition involving N enrichment led to biomass production which differed significantly with the control \((p < 0.05)\). However, in XX09, N-alone addition resulted in maximum growth response \((248.74 ± 14.97 \text{ µgL}^{-1})\) which was almost 2 times the control \((183.02 ± 28.31 \text{ µgL}^{-1})\) and the P-alone addition \((180.19 ± 18.19 \text{ µgL}^{-1})\). The growth response \((257.78 ± 12.54 \text{ µgL}^{-1})\) obtained from N&P simultaneous addition showed no significant difference with the N-alone addition but significantly differed with both control and P addition. This trend in growth responses was consistent through day 4, especially in the point XX09. The growth response observed in P-alone addition \((176.05 ± 5.45 \text{ µgL}^{-1})\) did not differ significantly with the control \((191.15 ± 17.50 \text{ µgL}^{-1})\) throughout the incubation (Fig. 1b). The result of the growth rate (Fig. 1c) showed that P enrichments across the studied points; XX01 \((-0.03 ± 0.01 \text{ µgL}^{-1}d^{-1})\), XX06 \((-0.01 ± 0.02 \text{ µgL}^{-1}d^{-1})\), and XX09 \((-0.06 ± 0.02 \text{ µgL}^{-1}d^{-1})\) did not influence primary productivity as the consistent negative rate of growth just like the control characterized all P enrichments. Conversely, N enrichments led to positive growth rate suggesting significant stimulation on the biomass production in summer induced by N dynamics in the XXB. The maximum growth rates \((0.11 ± 0.02 \text{ µgL}^{-1}d^{-1})\) for N alone and \((0.14 ± 0.03 \text{ µgL}^{-1}d^{-1})\) for NP contemporaneous enrichments were reported in XX06. The nutrient response pattern strongly revealed N-limitation indicating that N addition controlled the bulk of the growth in summer. This was, especially true for XX09 wherein N enrichment induced maximum productivity. The N-limited conditions in summer have been reported e.g., the large but shallow hyper-eutrophic Lake Taihu, China (Paerl et al., 2011) and XXB (Li et al., 2020). Under the productive period usually summer, N-limitation in the aquatic system is predominantly observed (Domingues et al., 2011; Yahdjian et al., 2011). Liikanen and Martikainen, (2003) and Rangel et al., (2012) have also indicated that high temperature is associated with ammonification and exacerbates nitrate reductions (denitrification) processes with greater Fe-bound phosphorus releases from the chemical weathering of rocks. Although points; XX01 and XX06 also demonstrated potentially N limited conditions, the magnitude of N limitation in the mainstream (XX09) was more prominent throughout the incubation. The large row-crop agriculture dominant in XX01 and XX06 over XX09 could have contributed to the considerable N replenishment in these points (Zhou et al., 2012). For example, a high concentration of DIN in the mouth of XXB and high \(\text{PO}_4^{-}\text{P}\) towards the upstream has been previously reported (Ye et al., 2007). It was further observed that P-
alone addition led to slight repression on growth from 191.15 ± 17.50 µgL⁻¹ in the control to 176.05 ± 5.45 µgL⁻¹ in the P-alone addition as incubation lasted through day 4. This suggested P replete condition in XXB during summer and excess P supply in summer would not influence primary productivity. Another possible reason could be the fact that N-fixation usually does not seem to be able to fully satisfy N limitations in rivers and streams when P is present over N (Dodds and Smith, 2016) indicating that excess of P further reduces N-availability with possible repression on growth of the high N-dependent primary producers. Again, a recent study reported repression on the algal growth, *Chlorella regularis* by a large excess of P (250 mg L⁻¹) under an N-limited condition (Fu et al., 2019). The significant biomass production in XX09 could be attributed to the water column nutrient concentrations (TN = 0.8 mgL⁻¹, TP = 0.07 mgL⁻¹) in the mainstream of the Three Gorges Reservoir (Li et al., 202; Nwankwegu et al., 2020) which have been considered far beyond the internationally recognized eutrophication thresholds (Eitzmann et al., 2009; Urquhart et al., 2017). Water with high TN and TP is flushed into the XXB tributary during the backwater phenomenon in peak rise seasons (Nwankwegu et al., 2019). Although, the spatial distribution of chl-a in Lake Poyang, the largest freshwater lake in Yangtze River, China apart from nutrient fluxes has been linked to the shade index (Wu et al., 2014). The Yangtze River Basin, particularly the XXB reaches is an open system with no significant shade index throughout the year (Nwankwegu et al., 2020b). This indicates that the nutrient variations induced by the TGR operations which significantly affect downstream N and P stoichiometry could be the major factor determining the spatial distribution of biomass as chlorophyll-a. The spatial chl-a distribution has also been attributed to the variations in the limiting factors induced by the concurrent hydrological modifications (Mangoni et al., 2017) and the interchanging hydraulic control due to damming (Nwankwegu et al., 2019). The effect of different nitrogen forms; Nitrate-nitrogen and ammonium-nitrogen, as well as phosphorus in separate combinations with Fe was further evaluated (Fig. 2). It indicated that growth responses varied considerably. The nutrient additions involving; NO₃⁻ + Fe and NH₄⁺ + Fe revealed no significant variation under 2 d incubation but differed significantly (p < 0.05) with the growth response obtained through P + Fe addition under the same condition and time. However, as incubation lasted through day 4, a growth response which significantly differed with NH₄⁺ + Fe was observed in the NO₃⁻ + Fe combined enrichment. This confirms the metabolic association between NO₃⁻ and Fe over NH₄⁺ and Fe in the aquatic ecosystem functioning (Nwankwegu et al., 2020b). The net growth rates reported in the combined enrichment NO₃⁻/Fe (0.139 ± 0.009 µgL⁻¹d⁻¹) and P/Fe (0.141 ± 0.03 µgL⁻¹d⁻¹) supported the preference of the combinations in promoting growth than the NH₄⁺/Fe affirming the key role of iron in the nitrate uptake by the majority of phytoplankton (Nwankwegu et al., 2019). This substantially corroborated recent reported that iron requirement is greatly influenced by the nitrogen sources and phytoplankton growing on NH₄⁺ have low Fe requirement than those on NO₃⁻ (Nwankwegu et al., 2020b). The possible reason could be attributed to the fact that the former is directly incorporated into amino acids while the latter has to be converted to NH4 (Berman-Frank et al., 2007; Schoffman et al., 2016) prior to assimilation thus the high energetic investment would only select a dominance of a few taxa, particularly the diatoms (Nwankwegu et al., 2019) which demonstrate obligate nitrate uptake. However, maximum growths, (45.25 ± 5.33 µgL⁻¹) in day 2 and (70.84 ± 3.26 µgL⁻¹) in day 4 were reported in P + Fe contemporaneous addition. A
previous study also reported the enhancement of cyanobacterial bloom biomass (CBB) through phosphorus enrichment in the eutrophic Lake Taihu, China (Chen et al., 2014). Besides, absolute cyanobacterial dominance is usually induced at low N:P ratio (Li et al., 2020; Nwankwegu et al., 2019). The cyanobacterial biomass shows great response to changes in concentration of P in both annual and monthly ranges (Ding et al., 2018). Further, Sondergarrd et al (2011) previously reported strong relationship of biomass production as Chl-a with TP over TN in the late summer bloom.

3.2 Seasonal nutrient dynamics

The nutrients concentrations (Fig. 3) across the four seasons showed strong variations. While the seasonal variations in TN did not show a significant difference among seasons, the seasonal variations in the TP revealed a significant difference (p < 0.05) among seasons with the least TP concentrations observed during autumn (0.16 ± 0.11 µgL⁻¹) and spring (0.10 ± 0.00 µgL⁻¹) suggesting the limitation by P in both autumn and spring. This depicts that stricter N or N&P reductions would be crucial for lasting bloom control, especially during autumn and spring in XXB. Although, studies had indicated that all nutrient depletion may not essentially define limitation as phytoplankton often build up their intracellular nutrient pools via luxury consumption (Li et al., 2020; Domingues et al., 2017) for utilization when nutrient gradient tends low in the ecosystem. The maximum concentrations in TP were observed during summer (0.93 ± 0.13 µgL⁻¹) and winter (0.70 ± 0.092 µgL⁻¹). The seasonal dynamics in the TN/TP ratio were; 12.37 ± 1.98 in autumn, 16.81 ± 2.43 in winter, 20.37 ± 0.19 in spring, and 6.44 ± 1.91 during summer. This indicates that the dynamics in the TN/TP stoichiometry is principally controlled by seasonal variability in the TP concentrations. Considering that Ding et al., (2018) had stated that TN/TP < 9 characterizes N limitation while TN/TP > 22.6 defines P-limitation, the TN/TP in the present study thus revealed strong N limitation in summer and P limitation in spring (Fig. 3a). However, the reliability of TN/TP ratios (stoichiometry) as an index for nutrient limitation has been previously questioned (Lv et al., 2011). The maximum concentration of NO₃ (2.98 ± 0.28 µgL⁻¹) and the least concentration in NH₄ (0.28 ± 0.09 µgL⁻¹) was reported in spring indicating a nitrate replete and ammonium deficit condition during spring bloom while the minimum concentration of NO₃ (0.81 ± 0.02 µgL⁻¹) was observed during summer. This further explains why in Fig. 2 above, Fe addition with NO₃ led to significant biomass production. The maximum concentration in NH₄ (1.08 ± 0.07 µgL⁻¹) was reported in winter probably reflecting the total absence of ammonium-dependent phytoplankton groups e.g., the Cyanophyta in winter (Zhou et al., 2012). The dissolved N (DTN) and P (DTP) also showed strong fluctuations across seasons. The maximum DTN concentration (1.58 ± 0.09 µgL⁻¹) was obtained in autumn and least in spring (Fig. 3b). It was, however, not surprising to observe a maximum concentration in DTP of 0.09 ± 0.01 µgL⁻¹ during summer. Statistics revealed that the annual fluctuations in the DTP differed significantly (p < 0.05) across seasons and the significantly elevated DTP in summer could be attributed to the high-temperature dependent P release from legacy stores (Li et al., 2020). Based on the DTN and DTP seasonal variations, it would be sufficient to understand why N-limitation characterizes summer bloom. The seasonal N limitation for harmful algal bloom has been attributed to the internal P loading from sediment (Ding et al., 2018). The high TP concentration in Lake Erie has been linked to the internal phosphorus loading from the profundal
sediment (Nürnberg et al., 2019). All forms of N are soluble thus no precipitation mechanism is available for N immobilization (Paerl et al., 2016) and an ecosystem N dynamic is greatly influenced by the phytoplankton uptakes (Nwankwegu et al., 2020a) in addition to natural processes of nitrification and denitrification. Although, significant N-cycling processes are often not possible under microcosm conditions (Domingues et al., 2011). The atmospheric deposition, run-off, and decomposition, which are directly dependent on temperature, pH, and rainfall are the few strategies that ensure ecosystem N replacement. However, the *Microcystis spp* which are exceptionally harmful, disrupt food web and generate hypoxia often dominate the summer bloom causing the populations of the N$_2$-fixers including the *Aphanizomenon, Nostoc, Anabaena*, and *Cylindrospermopsis* to be laid off the system (Li et al., 2020; Paerl et al., 2011). In this case, the protracted N utilization without replenishment and an increasing P supply from sediment and the geogenic resources e.g., phosphorus rocks can exacerbate a critical N-limitation in freshwaters. On the whole, the spatial nutrient response pattern during summer strongly reveals N-limitation indicating that N enrichment would greatly control growth while prolonging primary productivity in the entire system.

### 3.3. Seasonal dynamics in biomass production as chl-a

The phytoplankton biomass (Fig. 4) considerably varied across seasons. Each season showed peculiar responses on growth induced by the different nutrient enrichments. In autumn, nutrient additions involving N, P, and in combinations revealed biomasses as chl-a in N (48.47 ± 6.05 µgL$^{-1}$), P (59.76 ± 6.73 µgL$^{-1}$), and N + P (68.40 ± 5.73 µgL$^{-1}$). This indicated that all the nutrient enrichment stimulated growth responses which differed significantly with the ambient chl-a concentrations both in the initial and control conditions. Although nutrient promotions on growth by N and P separate additions during autumn showed no significant difference (p > 0.05), the nutrient response adequately showed P-limited than N limited growth condition. However, the XXB nutrient limitation pattern slightly differed from the previous study by Paerl et al., (2011) in a shallow eutrophic freshwater, Lake Taihu where P limitation was reported in winter-spring but it is important to recall that these two freshwater systems have different ecosystem behaviours both in depth, hydrodynamics, nutrient fluxes, and phytoplankton species structure. For example, a consistent P-limitation of phytoplankton growth in eight (8) deep (mean depth ranges 40 m to 107 m) Brazilian tropical hydroelectric reservoirs have been previously reported (Rangel et al., 2012). Again, the nutrient limitation is related to ecosystem local factors including Secchi depth, land use, hydrology, and catchment characteristics (Rangel et al., 2012). In this consideration, Lake Taihu (mean depth = 1.9 m) is a very shallow hyper-eutrophic system (Paerl et al., 2011; Xu et al., 2015). Based on the European Union Water Framework Directive stipulation, freshwater systems with average depths (mean depth > 15 m) is deep, systems with an average depth between 3 and 15 m are shallow while very shallow system show average < 3 m deep (Zou et al., 2020: Phillips et al., 2008), XXB with average mean depth = 39.6 m (Nwankwegu et al., 2020) is, therefore, a typical deep system. Consequently, the natural P-input into XXB associated with the estuarine/watershed upwelling would be essentially negligible, unlike Lake Taihu often characterized by large turbulent burst and sediment resuspension events (Wei et al., 2020; Li et al., 2018) that induce a constant internal P release (Ding et al., 2012). Internal nutrient loading is a potential process regulating phosphorus dynamics contributing up to 86% P reduction from legacy stores,
phytoplankton, chlorophyll-a, and cyanobacterial blooms (Radbourne et al., 2019). Qin et al., (2020) recently showed that while P limitation predominates deep lakes and reservoirs, N limitation predominates shallows lakes and reservoirs. This indicates that a deviant nutrient limitation pattern is possible and largely dependent on the water depth. In the same study, the authors argued therein, that the biogeochemical mechanisms associated with water depth essentially control the nutrient dynamics in the freshwater systems. It further demonstrated that in shallow systems usually characterized by mixing depth > maximum depth, sediment exchanges and water column influences are dynamic thus often exacerbate potential N loss (denitrification) and enormous P release from the legacy store through precipitation, leading to low N:P ratio and consequently elicits N limitation. Conversely, in the deep systems, which are characterized by mixing depth < mean depth, the hypolimnion boundary receive minimal turbulent/perturbation seasonally while the maximum hydrodynamic actions are concentrated on the epilimnion. The retarded N loss with the increased P loss often through sedimentation and immobilization directly triggers the elevated N:P ratio causing P limitation to prevail. The release of the high level of remobilized P from sediment which significantly encourages maximal primary productivity has been recently reported in the deep (max depth = 31 m; mean depth = 13.6 m) Rostherne Mere, Cheshire, U.K. (Radbourne et al., 2019). A significant positive correlation (r = 0.74, p < 0.01) between Secchi depth and chlorophyll-a (chl-a) has been previously reported in the deep (mean depth = 28 m) Çaygören Reservoir, Turkey (Celik, 2012). This invariably suggests that light availability which is critical to chlorophyll-a dynamics decreases with depth in the eutrophic freshwaters. The N&P simultaneous addition in autumn led to maximum growth with P potentially stimulating optimal growth than N in the combinations. It thus reveals that in the light of the nutrient structure, P-alone rather than the traditional association of N&P could drive to a logaritmic biomass production in XXB during autumn.

In winter, all the nutrient additions including N, P and their contemporaneous additions resulted in growth responses which did not differ (p > 0.05) with the control. It was evident to deduce that nutrient limitation did not characterize winter bloom in XXB indicating that the limitation could be attributed to other factors including the dramatically reduced photosynthetically active radiation (PAR) and water temperature. Consequently, ANOVA post hoc analysis revealed significant variations (p < 0.05) in the winter temperature (9.31 ± 1.13°C) relative the other seasons coupled with high dissolved oxygen (DO) of 11.39 ± 2.0 mgL⁻¹ in winter as shown in Table 1. A positive response of the ecosystem to the light enrichment particularly in winter has been previously reported (Domingues et al., 2017). Again, Tomasky et al., (1999) while studying nutrient limitation of phytoplankton growth in Childs River, an estuary in Waquoit Bay, MA, USA reported no response to nutrient additions during the colder months. The study thus attributed the restriction on the phytoplankton growth during months other than May-Aug growth to the constantly changing factors, such as light, temperature or a physiological mechanism. Ye et al., (2007) also reported temporary disappearance of bloom following a drop of temperature and precipitation in XXB. The present study reported similar ambient physicochemical parameter especially during spring (Table 1) as that of the previous study by Ye et al., (2007) in XXB 13 years ago with the mean values of water quality indices as; DO (mean = 11.81 mg/L), pH (mean = 8.64 mgL⁻¹), and temperature (mean = 14.94°C). The protracted stability in the climatic variables, particularly temperature suggests that the XXB eutrophic status over the
last 13 years are controlled by the direct impact of human activities while the climate change only contributes to the indirect effect.

In spring, a similar nutrient limitation characteristic as autumn was observed but the magnitude of biomass production in both seasons significantly differed (p < 0.05). In summer, nutrient limitation shifted to N as N-alone addition caused a growth response which did not differ significantly with N&P combined addition but differed significantly with both P-alone addition and the control. The N limitation reported in summer corroborated previous studies in the different freshwater systems (Paerl et al., 2011; Xu et al.; 2015). In Waquoit Bay, USA, the accumulation of phytoplankton biomass in brackish and saline water was limited by the supply of nitrate during warm months (Tomasky et al., 1999).

The effect of different nutrient combinations with the essential micro-nutrients including Fe (both alone and in combinations), Si, Mn, Zn, and copper showed strong variability to seasons (Fig. 5). The response on growth stimulation by Fe was significant in both spring and summer. Across the seasons, the trace metals interaction in concert with N and P only significantly promoted growth (56.73 ± 5.18 µgL⁻¹) in autumn while the culture involving the trace metals combination without N and P additions caused significant growth inhibition rather than stimulation in all seasons. The growth responses in Fe enrichments in spring and summer indicated strong Fe limitations while the Si enrichment in autumn and summer revealed potential scenarios of Si co-limitations as growth responses in systems involving Si enrichments led to biomass production as chl-a which differed significantly with the control (p < 0.05). The multiple trace metal enrichments only demonstrated strong positive growth stimulation in autumn with only a slight growth stimulation in summer. Based on the response of Fe enrichment in summer, it can be deduced that the presence of other trace metal (Mn, Zn, and Cu) significantly preempted the effective Fe activity in promoting growth in the Fe + Mn + Zn + Cu combined enrichments. On the whole, it can, therefore, be concluded that while the multiple trace metals are essential for driving primary productivity in autumn, it potentially caused inhibition of growth and repressed Fe activity on growth in the other seasons. Similarly, a recent study by Huang et al., (2020) reported an initial growth inhibition which was eventually followed by an exponential resumption of the growth towards the end of incubation by multiple trace metal enrichments during a bioassay experiment during autumn in XXB. The significant growth enhancement by Fe in summer could be attributed to the dramatically low ferrous concentration which was below its detectable limit as revealed by FAAS analysis. Conversely, our recent study reported significant Fe distribution of 0.62 ± 0.03 mgL⁻¹ in autumn, 0.06 ± 0.02 mgL⁻¹ in winter, and 0.004 ± 0.01 mgL⁻¹ in spring (Nwankwegu et al., 2020b). This confirms that XXB shows strong seasonal variability in Fe distribution. A significant yield in chl-a concentration (82.70 ± 4.01 µgL⁻¹) was observed in NPSi combined enrichment in autumn. The NPSi again relatively caused maximum growth stimulation (9.89 ± 1.65 µgL⁻¹) in winter indicating that Si was crucial for the phytoplankton species structure in both seasons. In spring, maximum growth responses were reported in both NPSi (15.76 ± 1.47 µgL⁻¹) and Fe-alone (16.02 ± 0.21 µgL⁻¹) cultures. In summer, significant growth stimulations were demonstrated in Si-alone (91.50 ± 7.66 µgL⁻¹) and Fe-alone (99.92 ± 6.35 µgL⁻¹) additions. Generally, XXB shows a
potentially eutrophic-hypereutrophic system across following the magnitudes of chl-a based on trophic state stipulations (Celik, 2012; OECD, 1982).

3.4. Seasonal taxonomic dynamics

Significant seasonal variabilities in the population of each taxonomic group were observed (Fig. 6). Five principal phytoplankton taxa notably; Cyanophyta, Baccillariophyta, Chlorophyta, Cryptophyta, and Pyrrophyta were identified in XXB although, in autumn, the community structure included a few populations of the Euglenophyta and Xanthophyta. The pattern of taxonomic dominance showed strong sensitivity to variations in season. Each season, therefore, selected the dominance of one or more taxa but not all at the same time except in autumn where all the taxonomic groups were represented in the largest amount relative to other seasons. This indicates that autumn condition supports a wide range of phytoplankton taxa. During autumn, the community structure revealed significant cell densities in both the Cyanophyta (3.8×10⁷ cellsL⁻¹) and Chlorophyta (3.3×10⁷ cellsL⁻¹). A relatively lower cell densities were observed in Bacillariophyta (1.00×10⁷ cellsL⁻¹), Cryptophyta (3.10×10⁶ cellsL⁻¹), and Pyrrophyta (2.5×10⁶ cellsL⁻¹). In winter, significant cell density was observed in the Bacilliarophypt (1.01×10⁷ cellsL⁻¹) with significant losses in cell densities observed in Cyanophyta (8.57×10⁴ cellsL⁻¹), Chlorophyta (1.3×10⁵ cellsL⁻¹), and Cryptophyta (1.04×10⁵ cellsL⁻¹) while the Pyrrophyta were exclusively dominated. In spring, the Cryptophyta (1.23×10⁷ cellsL⁻¹) demonstrated maximum cell density. Decreases in cell densities were reported in Bacillariophyta (5.40×10⁶ cellsL⁻¹), Chlorophyta (2.40×10⁶ cellsL⁻¹), and the Pyrrophyta (1.2×10⁶ cellsL⁻¹) with a total extinction of the Cyanophyta. In summer, an absolute dominance by the Cyanophyta (9.01×10⁷ cellsL⁻¹) was reported. The Chlorophyta (1.19×10⁷ cellsL⁻¹) was the next in terms of population strength indicating a slight similarity with the autumn bloom although the compositional turnover between the seasons varied considerably. The community structure in summer showed significantly low cell densities in the Pyrrophyta (5.85×10⁶ cellsL⁻¹) and Bacillariophyta (1.13×10⁶ cellsL⁻¹) with the total elimination of the Cryptophyta. The seasonal population dynamics could be attributed to the characteristic seasonal fluctuations in the nutrient concentrations, as well as the high taxonomic variability. The phytoplankton community structure is essentially controlled by the frequency in nutrient rate and supply ratios (Nwankwegu et al., 2019). The nutrient concentration dynamics including TN, TP, and the nitrogen oxidation state (NO₃ & NH₄) fluxes greatly affect phytoplankton community structure in the aquatic system (Harris et al., 2017). The total loss of the Cyanophyta and high Cryptophyta in spring could be linked to the significantly low concentration of NH₄ while the nitrate replete condition prevailed. Previous studies have reported the preferential uptake of NH₄ by the Cyanophyta during bloom (Xu et al., 2013; Xu et al., 2015; Schoffman et al., 2016). The Cyanophyta show high competitiveness for NH₄ utilization while the diatoms demonstrate high competitiveness for NO₃ uptake (Harris et al., 2017). The significantly high population turnover in the Bacillariophyta in winter confirms the absolute tolerance of diatoms to cold spells and the high mixing regimes that characterize winter bloom in XXB following the backwater intrusion from the mainstream TGR during the peak rise season. Absolute ecosystem control by the Bacillariophyta, dominated by the Cyclotella in 15 subtropical, urban shallow lakes in Wuhan China has been previously reported (Lv et al.,
It has also been demonstrated that the Bacilliarophyta e.g., diatoms prefer the system fast and well mixed while the Cyanophyta prefer it slow, hot and significantly stratified thus dominate summer blooms (Nwankwegu et al., 2019). Again, the diatoms possess the ability to thrive under low light and temperature thus constitute the dominant species in both lentic and lotic systems during the winter bloom (Paerl et al., 2016).

3.5. Species transitions across seasons

Species/genera dominance varied strongly across seasons (Table 2). The compositional turnover among the taxonomic groups revealed that variability in the seasons played a significant role in the seasonal species transitions. There is no stability in the species compositional pattern except in the taxon, the Cryptophyta where a stable genera/species distribution dominated (d) by *Chroomonas acuta* prevailed during autumn, winter, and spring with a sharp species decline in summer where single dominance (d) of species, *Cryptomonas erosa* was reported. The significant prevalence of the Cryptophyta dominated by the *Chroomonas acuta* (D) was reported in spring. In the Bacillariophyta, the genera, *Cyclotella* dominated (d) across seasons except for summer where only two species of the group; *Synedra acus* and *Cocconeis sp* survived with species dominance (d) controlled by the later. While no members of the Cyanophyta was observed in winter, the *Cyclotella* was the dominant (D) genera in the entire community structure. The Chlorophyta showed maximum genera/species composition, particularly in autumn and winter with regime shift in the intra-species dominance (d) which alternated among the members of the taxon across the seasons from *Scenedesmus spp* in both autumn and spring to *Ulothrix sp* in winter, and the *Actinastrum fluviatile* during summer. In the Cyanophyta, species dominance was controlled by the *Microcystis spp* both within the taxon (d) and among community structure (D) in autumn and summer. The phytoplankton community structure in both winter and spring did not include any member of the Cyanophyta. The possible reasons could be the significantly low temperature in winter (Paerl et al., 2016) and the ammonium-nitrogen (NH$_4^+$) deficit condition in spring which the Cyanophyta preferentially utilize (Li et al., 2020; Xu et al., 2013). Although the previous study by Cardoso et al., (2012) attributed the change in phytoplankton community to the local ecosystem factors, the seasonal variability and nutrient dynamics are the principal factors that strongly control the phytoplankton community shift in XXB. The loss of the several species of other taxa during summer could be linked to the absolute dominance of the toxic, hypoxia generating, and food web disrupting *Microcystis spp* (Nwankwegu et al., 201; Paerl et al., 2011). The cyanobacteria can swim along the water column dominating the epilimnion and hypolimnion (Li et al., 2020; Nwankwegu et al., 2019). The development of other phytoplankton taxa including the eukaryotic organisms in the photic zone is preempted by a significant turbidity and hypoxia associated with cyanobacterial dominance under intense bloom (Sanseverino and Conduto, 2016). The maximum dominance of *Microcystis spp* in summer could be attributed to the significantly low TN/TP. The low TN: TP (N- limitation) usually result in cyanobacterial dominance in the eutrophic freshwaters (Harris et al., 2017).

Conclusion
The present study reports the spatial and seasonal distribution of chlorophyll-a (chl-a) induced by the remarkable fluctuations in the different nutrient concentrations across the four seasons; autumn, winter, spring, and summer in XXB. Our study proves that the mainstream reservoir is severely eutrophic with significant biomass production as chl-a during the intense summer bloom than the rest of the reaches. The phytoplankton community structure showed strong responsiveness to the seasonal variability with a regime shift from Cyanophyta, dominated by the *Microcystis spp* in both autumn and summer. The study reveals relative stability in the concentrations of TN, while the TP, NO₃, NH₄, DTN, and DTP showed significant seasonal variabilities. An NH₄ deficit and NO₃ replete condition was reported during spring which probably initiated the total extinction of the Cyanophyta that show absolute preference to ammonium-nitrogen. It reckons that nutrient inputs regulations in the surrounding counties; Zigui and Xingshan in Xiakou Town though potent in the proximal tributaries would not be adequate to address the current state of bloom in the mainstream. It, therefore, points to the need for an extended mitigation design to the highly eutrophic mainstream which recharges the XXB through the backwater intrusion during the peak rise seasons. The study thus proposes broad monitoring programs while emphasizing that sectional ecological assessment would not effectively characterize the whole freshwater systems and recommend such extended assessment convention in the similar reservoirs and tributaries worldwide facing similar anthropogenic pressures.

**Declarations**

**Ethical Approval and consent to participate**

Not applicable to this submission

**Consent to Publish**

Not applicable

**Authorship’s contributions**

**Amechi S. Nwankwegu:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. **Yiping Li:** Funding acquisition, Supervision. **Lei Zhang, Deti Xie:** Visualization, Supervision, Writing - review & editing. **Yanan Huang:** Formal analysis, Visualization. **Eyram Norgbey:** Formal analysis, Visualization. **Hans W. Paerl:** Visualization, Supervision, Writing - review & editing

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Competing interest

Authors declare that they have no competing interests.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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Tables

**Table 1** Seasonal dynamics in ambient XXB physicochemical characteristics (mean ± standard deviation)

| Parameter  | Autumn 2018 | Winter 2018/2019 | Spring 2019 | Summer 2019 |
|------------|-------------|------------------|-------------|-------------|
| WT (°C)    | 25.20 ± 1.20ₐ | 9.31 ± 1.13ₐ    | 16.30 ± 1.43ₐ | 28.20 ± 2.0ₐ |
| pH         | 9.20 ± 0.80ₐ  | 7.94 ± 2.0ₐ    | 8.62 ± 1.52ₐ | 9.44±0.06ₐ  |
| DO (mgL⁻¹) | 9.54 ± 0.23ₐ  | 11.39 ± 2.0ₐ   | 7.71 ± 0.69ₐ | 8.1±0.11ₐ  |
| EC (mScm⁻¹) | 0.30 ± 0.01ₐ | 0.36 ± 0.04ₐ  | 0.42 ± 0.32ₐ | 0.26±0.06ₐ |

Note: WT = water temperature, DO = dissolved oxygen, EC = electrical conductivity. Same parameters with different superscripts differ significantly (P > 0.05) across seasons.

**Table 2** Seasonal compositional dynamics and dominance within genera/species and among the phytoplankton community structure
| Taxon | Season | Bacil. | Chlor. | Cyan. | Pyrro. | Crypt. |
|-------|--------|--------|--------|-------|--------|--------|
| **Austrias** | | | | | | |
| *Anabaena circinalis* | | | | | | |
| *Cyclotella* | | | | | | |
| *Melosira granulata* | | | | | | |
| *Streptococcus* | | | | | | |
| *Nitzschia* | | | | | | |
| *Navicula* | | | | | | |
| **Wapag** | | | | | | |
| *Cymbella* | | | | | | |
| *Nitzschia* | | | | | | |
| *Anabaena circinalis* | | | | | | |
| *Amphora* | | | | | | |
| *Cyclotella* | | | | | | |
| *Melosira granulata* | | | | | | |
| *Streptococcus* | | | | | | |
| *Nitzschia* | | | | | | |
| *Navicula* | | | | | | |
| **Spring** | | | | | | |
| *Cyclotella* | | | | | | |
| *Melosira varians* | | | | | | |
| *Bacillariophyta* | | | | | | |
| *Sympodium* | | | | | | |
| *Nitzschia* | | | | | | |
| *Melosira granulata* | | | | | | |
| **Summer** | | | | | | |
| *Nitzschia* | | | | | | |
| *Coscinodiscus* | | | | | | |

*d dominant genera/species within taxon, D dominant genera/species among the entire community. Bacil = Bacillariophyta, Chloro = Chlorophyta, Crypto = Cryptophyta, Pyrro = Pyrrophyta

**Figures**
Figure 1

Relative phytoplankton biomass (chlorophyll-a, µgL⁻¹) responses in day 2 (a), day 4 (b), and net growth rate (µgL⁻¹d⁻¹) (c) in bioassays conducted in summer, 2019 at points XX01, XX06, and XX09. Control chlorophyll-a concentration is shown for each point. Responses were for 4-day incubations in summer. Mean values are shown. Error bars represent ±1SD of triplicate samples. D2 to D4 means incubation time in day. Differences among treatments across points are shown based on ANOVA post hoc tests (a > b > c; p < 0.05).
Figure 2

Phytoplankton biomass (chlorophyll-a, µgL-1) responses (a) and net growth rate (µgL-1d-1) (b) induced by different nitrogen forms (nitrate-nitrogen and ammonium-nitrogen), and phosphorus (P) combination with iron (Fe) during bioassays conducted in summer, 2019 at point XX06. Responses were for 4-day incubations in summer. Mean values are shown. Error bars represent ±1SD of triplicate samples. D2 to D4 means incubation time in day. Differences between treatments are shown based on ANOVA post hoc tests (a > b > c; p < 0.05).

Figure 3

Dynamics in nutrient concentrations in XXB in autumn 2018, winter, 2018, spring, 2019, and summer, 2019 at point XX06. TN = Total nitrogen, TP = Total phosphorus, TN/TP = Nutrient ratio, NO3 = Nitrate, NH4 = ammonium (a), DTN = Dissolved total nitrogen, DTP = Dissolved total phosphorus (b).
Figure 4

Seasonal fluctuations in phytoplankton biomass (chlorophyll-a) responses in bioassays conducted in autumn 2018, winter, 2018, spring, 2019, and summer, 2019 at point XX06. Nutrient additions revealed nutrient limitation shift pattern over a one-year period. Incubations lasted for 4 d in each season. Mean values are shown. Error bars represent ±1SD of triplicate samples. Differences between treatments are shown based on ANOVA post hoc tests (a > b > c; p < 0.05).
Figure 5

Dynamics in phytoplankton biomass (chlorophyll-a) responses across seasons induced by bioassays involving micro-element additions conducted in autumn 2018, winter, 2018, spring, 2019, and summer, 2019 at point XX06. Nutrient additions revealed either trace metals stimulatory or inhibitory to growth across seasons. Incubations lasted for 4.d in each season. Mean values are shown. Error bars represent ±1SD of triplicate samples. Differences between treatments are shown based on ANOVA post hoc tests (a > b > c; p < 0.05). Ms composed of Fe, Zn, Mn, and Cu

![Cell density graph](image)

Figure 6

Seasonal transition in dominance by the representative phytoplankton groups in XXB in autumn 2018, winter, 2018, spring, 2019, and summer, 2019 at point XX06. Cyano = Cyanophyta, Bacili= Bacilliarophyta, Chloro = Chlorophyta, Pyrro = Pyrrophyta, and Crypto = Cryptophyta

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