Nutrient addition bioassay and phytoplankton community structure monitored during autumn in Xiangxi Bay of Three Gorges Reservoir, China

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HIGHLIGHTS
- Episodic HABs events are driving by increasing nutrient budget and constantly changing climate.
- Increasing nutrient paradigm greatly affects biological diversity and exacerbates competition and succession.
- Trace metal addition causes a community transition from Cyanobacteria to Chlorophyte dominated system.
- Combined N, P and Si addition stimulates growth rate and optimizes biomass production (as chla).

ABSTRACT
The increasing freshwater ecosystem nutrient budget is a critical anthropogenic factor promoting freshwater eutrophication and episodic bloom of harmful algae which threaten water quality and public health. To understand how the eutrophic freshwater ecosystem responds in term of phytoplankton community structure dynamics to a sudden rise in nutrient concentrations, a microcosm study by nutrient addition bioassay was implemented in Xiangxi Bay (XXB) of Three Gorges Reservoir, China. Our results showed that dissolved trace elements supply adequately altered the phytoplankton community structure creating a regime shift from cyanobacteria-dominated to essentially Chlorophytes-dominated system, relative abundance (>70%). Combined N, P, and Si led to maximum growth stimulation accompanied by the highest chlorophyll yield (82.7 ± 14.01 µgL⁻¹) and growth rate (1.098 ± 0.12 µgL⁻¹d⁻¹). N separate additions resulted in growth responses which did not differ while P-addition differed significantly (p < 0.05) with the control justifying a P limited system. Si enrichment stimulated diatom growth, relative abundance (20.62%) and maximum utility rate (U Si = 83.37 ± 0.33%). This study also reveals that increasing nutrient loading from anthropogenic sources adequately decrease the ecological diversity (H < 1) and community overlap (CC ≤ 0.5) intensifying competition and succession which then select the fast-growing taxa to dominate and expand. Result points to the need for
1. Introduction

Harmful algal blooms (HABs) are a pervasive environmental problem with an increasing trend in both severity and geographic range regularly induced by several factors. Anthropogenic nutrient input into freshwater-marine continuum has dramatically increased the current state of episodic HABs (Nwankwegu et al., 2019). The constantly changing climate, extreme hydrologic events and weather conditions such as rainfall, droughts, cyclones, and heavy storms, as well as atmospheric deposits, largely impact HAB and nutrient dynamics (Huo et al., 2019; Li et al., 2019; Pesce et al., 2018; Val et al., 2016). In the past, the phosphorus (P) was recognized as the only nutrient ultimately controlling freshwater eutrophication and bloom accretions (Paerl, 2008). A critical consideration was consequently given to P-alone input reduction in an attempt to mitigating the episodic outbreak of HABs in most aquatic systems. This management approach stemmed from the initial conclusion that the process of denitrification was sufficient to keep in check the excess N-input into freshwater systems.

Subsequently, several studies essentially demonstrated that varying phytoplankton taxa respond differently to a wide range of nutrients varieties including N, P and most micronutrients in concert (Binding et al., 2018; Coronado-fracono et al., 2018; Kumar et al., 2018; Wu et al., 2018) and a single nutrient management protocol would no longer guarantee a lasting solution to the HAB outbreaks. Frequently, cases of species-specific nutrient metabolism occur, significantly providing an advantage for ecosystem dominance and while some algal species are sensitive to P limitation others remain unaffected except N becomes limiting (Franklin et al., 2018, 2018; Silkin et al., 2019; Weisse et al., 2016). The sensitivity of phytoplankton community structure to the increasing nutrient concentrations in aquatic systems has received much attention (Klose et al., 2015). This outcome raised global concern on the need to resolve the relationship between phytoplankton structures and the aggregate nutrient disturbances rather than just P in the eutrophic freshwater systems. The nutrient budget in a eutrophic system essentially cut across the macronutrients (N & P) including their dissolved, oxidized and reduced forms, and the micronutrients notably; iron (Fe), silicon (Si), manganese (Mn), zinc (Zn), and copper (Cu). Thus a comprehensive study on nutrient pool driving phytoplankton dynamics and bloom accretions should take into cognizance the extended nutrient pool.

In the Xiangxi Bay (XXB), a tributary in the Yangtze River, China (the world’s third-largest river), severe eutrophication and harmful algal bloom has remained unabated since the impoundment of the world’s largest hydroelectric project, the Three Gorges Dam (TGD). Several studies while attempting to provide a sustainable bloom management strategy have evaluated the hydrodynamic influences on the bloom condition including current characteristics (Yang et al., 2018), vertical mixing (Johnson et al., 2012), isotopic nutrient distributions (Yang et al., 2015), as well as sediment P release (Zhijing et al., 2012). However, the information on how sudden repletion in the critical nutrients controlling eutrophication induced by anthropogenic actions from mining activities in the surrounding towns affect the phytoplankton community structure has not been considered. The relationship between macromolecules and phytoplankton structure dynamics as they affect the aquatic ecosystem functioning are often very difficult to recognize and no study of such at present has been carried out in the eutrophic XXB. Also, since aquatic nutrient budget shows characteristic dynamic fluctuations, the study on the phytoplankton responses to the sharp increase in the nutrient pool would adequately complement the strategies for bloom control. This could help in enacting a prudent mitigation step that can guarantee a lasting solution to the expanding phytoplankton bloom in the tributary. Hence, the aim of the present study was to examine the dynamics in the phytoplankton community structure under abrupt nutrient repletion while elaborating macro-micronutrient synergy on growth stimulations through the deployment of field microcosms study involving nutrient addition bioassay. It also evaluated the dominance and pattern of taxonomic shift controlled by nutrient dynamics. The sensitivity of a bioassay informed its relevance in the present study since it is used to track the immediate assessment of nutrient-algae interactions through rapid biomass growth responses under controlled conditions usually 3–5 days incubation. Further, we hypothesized that nutrient additions would significantly affect the phytoplankton community structure and ecological diversity in the eutrophic Xingxi Bay.

2. Materials and methods

2.1. Study area

Xiangxi Bay (XXB) is the largest tributary in the lower reach of Three Gorges Reservoir (TGR) Yangtze River, China, located within the latitude 31°04′–31°34′ N and longitude 110°25′–111°06′E. It has a length of 94 km, the average annual flow of 47.4 m³/s and the watershed area of 3099 km² (Gao et al., 2018). It is 32 km from Three Gorges Dam (TGD), and 38 km upstream of Yangtze River. It originates from Shennongjia National Park, Northwestern Hubei province, China. The X XB (Fig. 1) flows through Xingshan and Zigui Counties, north to south while it empties into the mainstream of TGR in Xiangxi Town (Yang et al., 2015). Just like Yangtze River, the largest river in Southeast Asia originating from Tibetan plateau, XXB possesses subtropical continental monsoon climate. The emergence of XXB in Xiangxi River is a direct consequence of hydraulic operations of TGR. A deep riverine bay emerged soon after the initial impoundment of TGR in 2003 creating a backwater which extended to 40 km when the reservoir was first filled to the maximum water level of 175 m in 2010 (Ji et al., 2017; Jiang et al., 2018; Zhu et al., 2013). The mainstream impoundment elevation alternates within 135 m, 145 m, 156 m and 175 m based on time of the year. Consequently, the hydrodynamics of XXB has remarkably changed in responses to the TGR operations. Notably, the bay is now characterized as a stable system similar to lakes with some defined limnetic characteristics including thermal stratification, long residence time and eutrophication, all of which are triggers of harmful cyanobacterial blooms (Xu et al., 2011). The hydrologic position of XXB is such that inflow discharge from upstream Yangtze River (38 km) has little or no hydrodynamic impact in the bay. The backwater intrusion from mainstream TGR happened to be the only recharging source and this hydrologic event is seasonal and occurs only in peak rise season following TGR closure. The annual average temperature and annual phosphorus load of XXB are 16.6 °C and...
29.78 tons, respectively with a residence time of 131 d. This situation has intensified nutrient saturation of this bay (Dai et al., 2010; Zhijing et al., 2012). The nutrient concentrations of the mainstream (TN = 0.8 mgL⁻¹, TP = 0.07 mgL⁻¹) have been considered far beyond the internationally recognized eutrophication threshold (Zhou et al., 2011). Most studies posited that the nutrients supply sources of XXB are that non-point source loads of TN and TP supply from upper XXB tributary which constitutes 61% and 40% of TN and TP, respectively (Yang et al., 2015). Furthermore, Shu Kongping chemical and mining industrial area located in Xiakou Town, the end of backwater zone of XXB with a characteristic leaching behaviour of waste rocks is another major source of phosphate rocks in XXB watershed (Jiang et al., 2016). Consequently, an influx of ≥22.5% yellow phosphorus into XXB through ruff-off has been attributed to the commercial activities of the chemical industry in Gufu Town (Huang et al., 2012). These constant nutrient supplies are a key to the factors exacerbating eutrophication and bloom phenomenon in the bay and this water quality problem which ultimately threatens public health needs urgent attention.

2.2. Nutrient addition bioassay

A microcosm nutrient addition bioassay was conducted in XXB during autumn (August 19 – September 12, 2018) to rapidly assess the phytoplankton-nutrient interactions, utilization as well as nutrient fate to the natural algal community structure. A surface water sample (0.2 m) was collected from point XX05, the middle and representative of XXB into a big plastic container (300 l, 0.01 N HCl-sterilized and XXB water-rinsed) used for all assay. To ensure even distribution of nutrients and phytoplankton species in the water, a stirrer was used and continuous stirring provided while 4 l aliquot was dispensed into acid (0.01 HCl) and then XXB water-washed 5-l polyethylene Cubitainers. The fact that Cubitainers are chemically inert, foldable, unbreakable, transparent thus transmit ≥80% PAR necessitated its choice in the present study (Paerl et al., 2011; Paerl and Bowles, 1987; Xu et al., 2015). Prior to the bioassay study, XXB water sample was analyzed for ambient nutrients, chlorophyll (Chla) and physicochemical characteristics such as dissolved oxygen (DO), electrical conductivity (EC), pH and temperature. A total of eight treatments including the control (no enrichment) were conducted (Table S1). These included (1) N addition (+N), (2) P addition (+P), (3) Si addition (+Si), (4) N and P addition (N + P), (5) N, P and Si addition (N + P + Si), (6) metal addition (Fe, Zn, Cu, and Mn), (7) consortium involving the equivalent concentrations of all as in single microcosms (N + P + Si + metal). N was supplied as KNO₃, P as K₂HPO₄, Si as K₂SiO₃, and metals (Fe, Zn, Cu, and Mn) as FeCl₃, ZnSO₄, CuSO₄, and MnSO₄, respectively. The final concentrations of all nutrients were
1.0 mgNL⁻¹ for N, 0.1 mgPL⁻¹ for P, 6 mgSiL⁻¹ for Si, and 0.1 mgL⁻¹ each for the trace elements (metals). These concentrations approximated the baseline values of the forms of the nutrients in X XB as quantified prior to the start of the experiment (Table 1). In order to satisfy the incidence of carbon limitation as incubation lasted in each microcosm, all treatments received 10 mgL⁻¹ KHCO₃ supply (Xu et al., 2015). All treatments were conducted in triplicates which generated a total sample unit n = 24. Following nutrient enrichments, Cubitainers were incubated for 4 days at the XX05 platform while placing them on floating frames allowing for natural light, temperature and wave action (Pae rl et al., 2011; Xu et al., 2015). For logistical reasons, difficulty in navigating X XB (XX05) and the attempt to ensure the natural environment for phytoplankton growth, a bioassay was deployed in situ. To prevent photo-inhibition during the incubation, a layer of neutral density screening was placed over the frames (Pae rl et al., 2011) which reduced the incident radiation by 30% (Xu et al., 2013) as an increased ultra-violet radiation (UVR) can elicit negative effect on some phytoplankton taxa e.g. cyanobacteria (Domínguez et al., 2016) which are the dominant species during autumn in X XB. The Cubitainers were sampled for nutrients and chla concentrations at 1–2 days intervals.

2.3. Water sample analyses

In situ measurements of pH, temperature, dissolved oxygen (DO) and electrical conductivity (EC) were performed using multi-sensor Yellow Spring Instrument, sonde (Model 6600, YSI Inc., http://www.ysi.com). Water samples from each microcosm were analyzed for total nitrogen (TN), total dissolved nitrogen (TDN), ammonium (NH₄-N), nitrate-nitrogen (NO₃-N), silicate, total phosphorus (TP), total dissolved phosphorus (TDP) and chlorophyll. TN, TDN, TP, and TDP were analyzed by combined persulfate digestion (Pae rl et al., 2011) and further followed by spectrophotometric analysis for nitrate-nitrogen (NO₃-N). Ammonium nitrogen (NH₄-N) was analyzed using the indophenol blue method.

Pre-cleaned Whatman GF/F filters were used to filter water samples for dissolved nutrients analysis and acidified with trace metal-clean HCL (Baseline; Seastar) to pH 2. The total dissolved Fe (TDFe), total dissolved zinc (TDZn), total dissolved copper (TDCu) and total dissolved manganese (TDMn) concentrations in the filtrate were measured using inductively coupled plasma/mass spectrometry (ICP/MS) with detection limits of 2 lgL⁻¹ for Fe, 0.021 lgL⁻¹ for Mn, 0.01 lgL⁻¹ for Cu and 0.1 lgL⁻¹ for Zn (Xu et al., 2013). Biological parameters including chlorophyll a (Chl a) determination and phytoplankton identification also followed. Briefly, a water sample from each treatment, 150 mL for Chla analysis was filtered onto 25 mm GF/F filters, gently folded and blotted dry, placed in foil wrappers and then kept frozen (− 80 °C) until analysis (Zhou et al., 2016). After extraction in 90% hot ethanol, chla concentration was quantified spectrophotometrically (Pae rl et al., 2015). Separate 450 mL aliquots from each treatment for phytoplankton identification were fixed with Lugol’s iodine solution (2% final concentration) and settled for 48 h and identified (Pae rl et al., 2015). Algal cell density was microscopically measured using a Sedgwick - Rafter counting chamber at magnifications of 320–400 x. Species of phytoplankton were identified following Xu et al. (2013) and grouped into their corresponding taxa. The growth rate (μ) was estimated for each treatment following the exponential growth equation (Xu et al., 2015):

\[
\mu = \ln(X_2-X_1) / (T_2-T_1)
\]

Where X₁ is the concentration of Chl-a at the beginning of the initial time interval (T₁), and X₂ is the concentration of Chl-a for (T₂). The T₁ and T₂ are the incubation time intervals. To track changes in phytoplankton community structure as well as the extent of community similarity/overlap arising from nutrient additions/disturbances, biological diversity indices including Shannon index (H), Simpson index (D), and Sorenson’s coefficient (CC) were adopted with the formulae stated below;

\[
H = - \sum_{i=1}^{s} p_i \ln p_i
\]
\[
D = \frac{1}{\sum_{i=1}^{s} p_i^2}
\]

where \(H = \) Shannon index, \(D = \) Simpson index, \(p_i = \) the proportion (n/N) of a particular phytoplankton group (n) divided by the total population of groups (N), \(\ln = \) the natural log, \(\Sigma = \) sum of the calculations, and \(s = \) number of species.

\[
(CC) = \frac{2C}{S_1 + S_2}
\]

where \((CC) = \) Sorenson’s coefficient, \(C = \) the number of phytoplankton species the two communities have in common, \(S_1 = \) Total number of species found in community 1 (control), and \(S_2 = \) Total number of species found in community 2 (treatments) calculated individually for + N, +P, +Si, N + P, N + P + Si, metals, and N + P + Si + metals microcosms and each compared with the control.

2.4. Statistical analysis

The mean of replicate data was used for all measurements and standard deviations (error bars) analyzed with origin software. The nutrient concentrations, the phytoplankton cell density/relative abundances, and growth responses (chl-a) within replicates were statistically compared using t-test or ANOVA Post Hoc Multiple comparisons for treatment mean performed by Tukey’s least significant difference procedure (5% and 1% levels of significance). The effects of exogenous nutrients on phytoplankton community structure with incubation time were measured by grouping algal species into seven (7) phytoplankton taxa. The Cyanophyta, Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water temperature (°C)</td>
<td>28.0 ± 2.00</td>
</tr>
<tr>
<td>Depth (m)</td>
<td>39.00 ± 1.0</td>
</tr>
<tr>
<td>pH</td>
<td>9.20 ± 0.80</td>
</tr>
<tr>
<td>DO (mgL⁻¹)</td>
<td>11.54 ± 0.23</td>
</tr>
<tr>
<td>EC (mScm⁻¹)</td>
<td>0.30 ± 0.01</td>
</tr>
<tr>
<td>TN (mgL⁻¹)</td>
<td>1.49 ± 0.31</td>
</tr>
<tr>
<td>DTN (mgL⁻¹)</td>
<td>0.86 ± 0.03</td>
</tr>
<tr>
<td>NH₄-N (mgL⁻¹)</td>
<td>0.21 ± 0.01</td>
</tr>
<tr>
<td>NO₃-N (mgL⁻¹)</td>
<td>0.42 ± 0.06</td>
</tr>
<tr>
<td>TP (mgL⁻¹)</td>
<td>0.13 ± 0.01</td>
</tr>
<tr>
<td>DTP (mgL⁻¹)</td>
<td>0.03 ± 0.01</td>
</tr>
<tr>
<td>DSI (mgL⁻¹)</td>
<td>4.72 ± 0.05</td>
</tr>
<tr>
<td>DFe (mgL⁻¹)</td>
<td>0.62 ± 0.02</td>
</tr>
<tr>
<td>DMn (mgL⁻¹)</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>DZn (mgL⁻¹)</td>
<td>0.06 ± 0.03</td>
</tr>
<tr>
<td>DCu (mgL⁻¹)</td>
<td>0.004 ± 0.00</td>
</tr>
<tr>
<td>Chla (mgL⁻¹)</td>
<td>16.20 ± 2.01</td>
</tr>
</tbody>
</table>

Note: DO = dissolved oxygen, EC = electrical conductivity, and D = total and dissolved nutrient forms respectively.

Table 1: The ambient X XB physicochemical and biological characteristics.
Chlorophyta, Bacillariophyta, Cryptophyta, Pyrrophyta, Xanthophyta, and Euglenophyta which were the dominant taxa during the present study. The temporal variations in the relative abundance of phytoplankton based on chl a concentration were evaluated from the cell density of the individual phytoplankton taxa identified. The nutrient utility rates (U) were calculated by estimating the difference between initial nutrient concentration and minimal nutrient concentration in the different bioassay enrichments.

3. Results and discussion

3.1. The ambient XXB physicochemical and biological conditions

The initial properties of XXB surface water prior to bioassay are presented in Table 1. The tributary bay at the time of the study showed ambient temperature characteristics (WT = 28 ± 2 °C). The water was considerably clear at some depth, with average depth 39 0 ± 1.0 m and DO (11.54 ± 0.23 mg L⁻¹). The pH was slightly alkaline which did not significantly change (p > 0.05) throughout the study. The system showed TN/TP ratio of 11.50 (TN = 1.49 ± 0.31 mg L⁻¹, TP = 0.13 ± 0.01 mg L⁻¹). This indicates that the system is not N-limiting as a critical N-limiting system has a TN/TP < 9 (Ding et al., 2018). The dissolved inorganic nitrogen growth demand showed strong variations between NH₄-N and NO₃-N assimilations under eutrophic condition. The concentration of NO₃-N (0.42 ± 0.06 mg L⁻¹) was 2 times higher than NH₄-N (0.21 ± 0.01 mg L⁻¹). The total dissolved nitrogen (TDN = 0.86 ± 0.03 mg L⁻¹) and chla concentration (16.2 ± 2.01 μg L⁻¹) were high to strongly suggest eutrophic freshwater scenario. The concentrations of all the dissolved trace elements including DSi, DFe, DZn, DCu and DMn were within the detectable limits (Table 1). The system is highly silicon and iron-enriched (DSi = 4.72 ± 0.05 mg L⁻¹) and iron (DFe = 0.62 ± 0.02 mg L⁻¹), respectively. The XXB demonstrated a typical nutrient sufficient system limited by P with substantially no evidence of limitation by N.

3.2. Nutrient addition bioassays

The TN and TP (Fig. 2) uptake rates across the treatments strongly varied relative to the control. The microcosms involving N alone and Si alone additions resulted in the TN disappearance (uptake) rates which did not differ significantly (p > 0.05) with the control. In general, the microcosm involving the contemporaneous N, P, and Si additions demonstrated significant (p < 0.01) TN uptake rate relative to the control while a significant reduction in TN uptake relative to the control was reported in the microcosm involving the trace metals (M alone) additions. As for P, the significant TP uptake rates were reported across all microcosms that received P augmentation either alone or in combinations with other nutrients. It is noteworthy to recognize that the nutrient disappearance rates could result from several mechanisms including excretion, nutrient regeneration, and uptake which could essentially not link directly to the phytoplankton intracellular nutrient transport (Domingues et al., 2011a, 2011b). The nutrient pool accumulation via the luxury intracellular nutrient uptake (Domingues et al., 2015, 2017) can significantly increase nutrient disappearance rates. Considering that TN refers to a wide range of N forms notably; the organic nitrogen, ammonia, nitrate, and nitrite, several N cycling processes including nitrification and denitrification can play a crucial role in TN fluctuations indicating that disappearance rates may not necessarily follow a regular pattern.

Specifically, an increasing concentration of NH₄-N has been attributed to bacterial decomposition of organic nitrogenous matter and animal excretion rather than nitrification alone (Domingues et al., 2011a, 2011b) and increase in concentration of NO₃-N through nitrification cannot be totally neglected since N-fixers automatically kick-off N fixation as N concentration depletes to satisfy a transit N requirement in the eutrophic system. However, this phenomenon is usually almost unlikely under microcosm scenarios (Domingues et al., 2011a, 2011b). As for P, apart from the inward P transport and assimilation through phytoplankton cell membranes, the disappearance rate could also be influenced by the high possibility of P to become immobilized (adsorption, entrapment etc.) in particles within the micro-environment especially the bloom scum. The P is essentially susceptible to the precipitation mechanisms that limit its bioavailability in the aquatic systems (Paerl et al., 2016) and the internal P loading from legacy stores (sediment or rocks) ultimately temperature-dependent (Ding et al., 2018; Paerl et al., 2016).

The concentrations of TDN and TDP were low in treatments without N and P additions respectively (Fig. 3). TDN slightly decreased from day 0 to day 4 in all microcosms, however, a significant decrease (p ≤ 0.05) in the concentration of TDP from day 1 to day 4 was reported. This shows high P requirement to sustain growth at this time and suggested that P could be limiting (Zhou et al., 2009). The utilization of NH₄-N, NO₃-N (Fig. S1) and SiO₂ (Fig. S2) also varied among the enrichment systems. In the early stage of the study (day 0), NH₄-N concentration was high and later decreased dramatically from day 1–4 indicating high NH₄⁺ requirement by the phytoplankton community. In contrast, NO₃ and SiO₂ changed relatively small throughout the study. This could be attributed to the catabolite repression that occurs wherein the concentrations of NH₄⁺ in aquatic systems usually result in slight repression on the assimilation of NO₃ for growth. Similarly, previous studies in the eutrophic system have reported differential utilization of inorganic nitrogen compounds (Domingues et al., 2011a, 2011b; Swarbrick et al., 2019). In freshwater systems and other coastal bodies, NH₄⁺ is recognized by most algal species particularly the Cyanophyta as the preferred N-source for growth (Xu et al., 2011; Paerl et al., 2011). However, NO₃ relative to SiO₂ showed dynamic fluctuation in the concentrations with time. The SiO₂ remained relatively stable throughout the incubation indicating that NO₃ is taken up by a larger population of phytoplankton taxa than SiO₂ which probably selected single or few algal taxa particularly the diatoms. For example, significant cyanobacterial
responses to N addition in the absence of Si has been previously reported (Domingues et al., 2011a, 2011b). The decrease in concentration with time is a function of increased utilization as it is unlikely that denitrification played a substantial role in NO$_3$ depletion in the confined system within the incubation time. This, however, confirms the postulation by Domingues et al. (2011a, 2011b) that NO$_3$ depletion is similar to uptake considering the less likelihood of nitrification occurring inside the microcosm. The diatom show obligate Si requirement and high preference to NO$_3$ but majority of phytoplankton taxa e.g. Cyanophyta also utilize NO$_3$ under NH$_4$ deficit and NO$_3$ replete condition provided the system is Fe sufficient (Xu et al., 2013). The present study reported that XXB is Fe enriched with extremely low NH$_4$ which progressively decreased from day 0–4 thus the diatoms NO$_3$ uptake may not exclusively dominate. Although, previous study by Domingues et al. (2015) stressed that significant nutrient utilization may not necessarily reflect growth both luxury consumption. The concentrations of dissolved trace elements (TDFe, TDZn, TDCu, and TDMn) were consistent with dynamics in DIP but not DIN (data not available).

Effective chla yield (Fig. 4) occurred in day 2 through day 4 and varied among the microcosms. The maximum chla yield (chla = 82.7 ± 14.01 µgL$^{-1}$) was obtained in day 2 in the culture with N + P + Si addition and was immediately followed by N + P addition (chla = 68.4 ± 27.73 µgL$^{-1}$). The poor chla yields of 7.23 ± 0.067 µgL$^{-1}$ and 10.5 ± 2.00 µgL$^{-1}$ were observed in metal-alone addition and N + P + Si + metal addition respectively. Addition of Si in N + P + Si resulted in growth responses 1.21 times (17.30%) and 2.18 times (54.20%) greater than N + P in day 2 and 4, respectively. There was, therefore, optimum growth stimulation following Si enrichment. Metal-alone addition resulted in dramatic inhibitory growth response which led to biomass production less than the control (chla = 13.13 ± 2.50 µgL$^{-1}$) in day 2. Sufficient to posit that Si addition showed considerable stimulation of phytoplankton biomass production in the enrichment system. However, in day 4, it was striking to discover that while the biomass...
production (as chla) in the other microcosms including the initial and control experienced remarkable decrease, the metal −alone addition (chla = 13.3 ± 6.34 μg L⁻¹) and N + P + Si + metal addition (chla = 56.73 ± 5.18 μg L⁻¹) both showed significant increase (p < 0.05) in the chla concentrations relative to day 2. Possible reasons could be due to (i) the decreased pH and DO as well as a slightly elevated EC (Fig. S3) in the +metal-alone addition microcosms. This could have induced phytoplankton growth suppression in day 2 (ii) presence of Cu²⁺ in both +metal-alone and N + P + Si + metal additions might have triggered short term algal toxicity and perhaps cell lysis which dramatically reduced the populations and selected for resistant species that quickly proliferated and dominated under nutrient abundance and less competition in day 4 (iii) processes of bio-sorption and bioaccumulation may have played a role in reducing the initial toxicity of Cu²⁺ as incubation lasted through day 4 (iv) nutrient depletion in the other microcosms except the cultures with metals additions following active growth/proliferation in day 2 could have caused attenuation in chla yields to warrant a sharp decline in biomass production (as chla) in day 4. Nutrient and chla concentrations in XXB show obvious variations and the magnitude of biomass production largely dependent on the ecosystem nutrient budget (Li et al., 2018). Change in phytoplankton community structure of eutrophic systems results from the availability of nutrients which induce both intra and inter-specific competitions allowing the r-strategies to dominate the system (Zhou et al., 2018). The operating physicochemical characteristics greatly affect nutrient assimilation across the concentration gradients during bloom while it essentially controls bloom dynamics and growth. In Fig. S3, an apparent linear relationship between DO and pH was demonstrated. The result showed that DO and pH relate inversely with electrical conductivity thus an increase in EC can lead to a sharp decrease in both pH and DO (Chakraborty et al., 2011) as seen in the +metal-alone microcosms. Variation in temperature had little effect on nutrient dynamics, although nutrient release and circulation in the freshwater system, as well as the consequent increase in overall concentrations, have been linked to the high-temperature regime (Ding et al., 2018). There was, therefore, no significant difference (p < 0.05) in temperature changes among microcosms. A relatively high temperature (T = 27.97 ± 0.15 °C) was observed in P-alone addition but chla yield in N + P + Si (T = 26.83 ± 0.15 °C) outperformed the former. This could hence indicate that the effect of temperature would be more pronounced in the presence of other factors essential for algal growth including nutrient availability (Gasparini et al., 2017). In order words, provided the temperature is optimal, little variation in temperature among systems would not warrant any significant change in biomass production except other essential growth factors are lacking in addition.

3.3. Phytoplankton dynamics and competition among microcosms

The study on algal species essentially revealed several phytoplankton species which were classified under seven different phytoplankton taxa. A total of six taxonomic groups; Cryptophyta, Chlorophyta, Bacillariophyta, Phryrophyta, Cyanophyta, Xanthophyta were observed in all the microcosms. An additional taxon, Euglenophyta, was also reported in cultures with N + P + Si and metal-alone additions (Fig. 5). Effect of exogenous nutrients on community structure varied with different phytoplankton groups. This was especially true for Cyanophyta, Bacillariophyta, and Chlorophyta. Absolute taxonomic dominance of green algae, diatoms, and cyanobacteria in the Guadiana River, Portugal has been previously reported (Domingues et al., 2012). In a different study, Domingues et al. (2014) reported a frequent dominance by the toxic species in the cyanobacterial bloom of the Guadiana River, Portugal. The use of N + P + Si and metal as nutrients resulted in community structure alteration which triggered the introduction of a new phytoplankton group, Euglenophyta in the treatment options. This suggests that both extremes of growth conditions i.e. either completely healthy (e.g. N + P + Si) or completely depressed (e.g. metal-alone addition due to Cu²⁺) could remarkably change the natural integrity of freshwater ecosystem to warrant species succession. During the episodic algal bloom event, the cyanobacteria dominate and massively proliferate while producing dense surface scum to intensify hypoxia (Paerl et al., 2011; Sanseverino and Conduto, 2016). Throughout the enrichment systems including the control, the toxin-producing, food web-disrupting, and hypoxia generating harmful cyanobacterial species, the Microcystis spp dominated the Cyanophyta population. Also included are few species of N₂-fixers such as Anabaena azotica Ley, Anabaena circinalis, Aphanizomenon flos-aquae, Oscillatoria tenuis, and Pseudoanabaena sp while the diatoms, Bacillariophyta included; Attheya zachari, cyclotella, Melosira granulata, Melosira granulata var.angustissima f.spirals
and Chou (2003) also reported that diatoms have obligate silicon requirement for growth. Silicon-enriched eutrophic systems optimally enhance growth, metabolism, cell division and DNA replication to warrant diatom dominance (Wu and Chou, 2003). In the metal-alone addition, the Chlorophyta showed very high metal tolerance (RA = 74.75%) whereas other phytoplankton groups including the Cyanophyta (RA = 8.31%) demonstrated huge susceptibility to metal inhibition. This could be as a result of the fact that most representatives of cyanobacteria are planktonic and may have been dramatically reduced by cell toxicity and possible lysis due to precipitation of Cu\(^{+2}\) during which only the toxic species like the Microcystis which are able to collapse its vacuole gained the escape advantage. The Microcystis spp dominate the epilimnion and hypolimnion by swimming along the water column (Sanseverino and Conduto, 2016). This interaction would also explain why the dominant species in N + P + Si + metals were the cyanophyta (RA = 71.61%) bulk of which are Microcystis spp. Primary productivity and cyanobacterial growth are influenced by iron availability and iron has been found crucial in the process optimization of N\(_2\) fixation by the diazotrophs via heterocyst activation (Sanseverino and Conduto, 2016). N + P + Si + metals enriched system demonstrated optimal growth characteristics irrespective of the presence of metals. It could be likely that the concentrations of Si and Fe (compositions of metals enrichment) initiated a synergy which essentially neutralized the inhibitory effect provided by Cu\(^{+2}\). The Euglenophyta, Xanthophyta, and Pryrophyta showed extremely low relative abundance (<1) in all treatments including the control. Exogenous nutrient addition, therefore, had little or no responses on the growth of these taxa across the microcosms. Growth stimulation and responses including algal species dynamics were ultimately more pronounced in the three major phytoplankton taxa, Cyanophyta, Chlorophyta and Bacillariophyta throughout the study period. The nutrient utility rate (U) and the maximal growth rate (G) among the competing phytoplankton taxa strongly varied (Table 2). The optimal utility rates were consistent with the phytoplankton preferences to nutrient uptake. Highest utility rates (U_Si = 83.37 ± 0.33%), (U_DIN = 84.53 ± 0.58%), and (U_DIP = 73.33 ± 0.10%) in cultures with Si, N and P-alone addition, respectively were reported. The nutrient utility rate (U_Si and U_DIP) in cultures with N-alone addition did not vary significantly with control. The day 1 showed highest growth rate (G = 1.98 ± 0.12 µL\(^{-1}\)d\(^{-1}\)) which progressively decreased from day 2 (G = 0.50 ± 0.21 µL\(^{-1}\)d\(^{-1}\)) through day 4 (G = 0.225 ± 0.18 µL\(^{-1}\)d\(^{-1}\)) in culture with N + P + Si supply. In contrast, cultures with metal-alone and N + P + Si + metal supply experienced changes in growth rates in the increasing order of magnitude with G increase from −0.748 ± 0.19 µL\(^{-1}\)d\(^{-1}\) to 0.319 ± 0.03 µL\(^{-1}\)d\(^{-1}\) in metal-alone addition and −0.134 ± 0.08 µL\(^{-1}\)d\(^{-1}\) to 0.888 ± 0.05 µL\(^{-1}\)d\(^{-1}\) in N + P + Si + metals addition. The low growth rate in day 1 and dramatic rise in day 4 adequately tallied with the relative abundance due to initial toxicity induced by metal components on phytoplankton taxa except the Chlorophyta which displayed extreme tolerance and significantly proliferated.

### 3.4. Phytoplankton community structure and diversity under nutrients disturbances

Biological diversity by Shannon index (H), shows that increasing nutrient disturbances ultimately decreases the phytoplankton diversity (Table 3). Phytoplankton communities in the oligotrophic aquatic system are usually in relatively steady-state and adequately dominated by slow-growing nutrient opportunists which possess high affinity for nutrient utility (Vallina et al., 2014). This would substantially explain why the diversity index (H = 1.023)
in +metal-alone addition is higher than the cultures with simultaneous N and P addition, as well as N + P + Si additions where optimum biomass productions (as chla) were reported. It could then be deduced that biomass production does not practically reflect the extent of biological diversity instead it could suggest the type of dominant phytoplankton during bloom. For instance, while some algal form blooms with extended biomass production, others such as diatoms and most taxa though could impact water physical quality (colour and pH) produce less biomass. In contrast, Simpson diversity index (D) followed a different trend with the highest value (39.58) of primary productivity in aquatic systems by Paerl et al., 2011. The shift in phytoplankton community structure and composition while N + P + Si + metal addition showed slight overlap suggesting relatively low similarity. This could have been due to the variations caused by metal inhibition which adequately altered community structure. Since the closer the value of CC is to 1, the more similar the communities tend to be. Previous works showed the actual Si: DIN characteristic of XXB which does not contain exogenous nutrients thus served as a typical representative of XXB primary productivity index. The Si: DIN ratio throughout the study ranged between 5.49 ± 0.00—4.190 ± 0.00 in the initial and 2.64 ± 0.90—2.60 ± 1.40 in the control. Primary productivity in XXB is, therefore, significantly high thus confirmed the eutrophic nature of the bay.

Table 2
Nutrients utility rate (U) and maximal growth rate (G) in all microcosms.

<table>
<thead>
<tr>
<th>Microcosm</th>
<th>4 Utility rate (% U)</th>
<th>Maximal growth rate G (μgL⁻¹d⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Uᵣ</td>
<td>U₊N</td>
</tr>
<tr>
<td>Control</td>
<td>54.50 ± 0.18</td>
<td>39.16 ± 0.09</td>
</tr>
<tr>
<td>+N</td>
<td>59.18 ± 0.31</td>
<td>84.53 ± 0.58</td>
</tr>
<tr>
<td>+P</td>
<td>81.71 ± 0.35b</td>
<td>45.50 ± 0.54</td>
</tr>
<tr>
<td>+Si</td>
<td>83.37 ± 0.03b</td>
<td>40.43 ± 0.13c</td>
</tr>
<tr>
<td>N + P</td>
<td>62.96 ± 0.15</td>
<td>55.00 ± 0.38</td>
</tr>
<tr>
<td>N + P + Si</td>
<td>74.30 ± 0.30b</td>
<td>64.51 ± 0.55</td>
</tr>
<tr>
<td>Metal</td>
<td>22.37 ± 0.15</td>
<td>39.16 ± 0.09</td>
</tr>
<tr>
<td>N + P + Si + Metal</td>
<td>59.79 ± 0.33b</td>
<td>79.09 ± 0.38b</td>
</tr>
</tbody>
</table>

4 Values are mean of three replicates ±S.D. Mean superscripts with different letters differ significantly (p < 0.05) among treatments. D = d – day.

Table 3
Community similarity/overlap, Shannon index (H) and Simpson index (D) for all microcosms.

<table>
<thead>
<tr>
<th>Microcosms</th>
<th>+N</th>
<th>+P</th>
<th>+Si</th>
<th>N + P</th>
<th>N + P + Si</th>
<th>+Metals</th>
<th>N + P + Si + metals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.99</td>
<td>0.73</td>
<td>0.85</td>
<td>0.46</td>
<td>0.37</td>
<td>0.39</td>
<td>0.61b</td>
</tr>
<tr>
<td>1.101</td>
<td>1.17</td>
<td>1.08</td>
<td>0.81</td>
<td>0.80</td>
<td>0.90</td>
<td>1.02</td>
<td>0.841</td>
</tr>
<tr>
<td>2.860</td>
<td>2.83</td>
<td>2.49</td>
<td>2.75</td>
<td>4.70</td>
<td>8.13</td>
<td>1.65</td>
<td>1.190</td>
</tr>
<tr>
<td>1058.40</td>
<td>1190.55</td>
<td>1585.47</td>
<td>1385.68</td>
<td>2754.46</td>
<td>3008.80</td>
<td>418.00</td>
<td>2634.30</td>
</tr>
</tbody>
</table>

Note: CC = Sorenson’s coefficient calculated relative to control microcosm.
H = Shannon index, D = Simpson index, N = Σ (sum) of n – Total phytoplankton community in each microcosm.
n = Number of each phytoplankton group in each microcosm.
4 CC = Strong community overlap/similarity, 3 CC = slight overlap, CC ≤ 0.49 = Low overlap.
4. Conclusions

The present study compares the responses of different phytoplankton communities in Xiangxi Bay to the increasing concentrations of N, P, Si, and some trace metals. It also quantified the biomass production (as chla) and estimated the nutrient appearance (uptake) rates with time. This provides comprehensive insights into underscoring the effect of increasing anthropogenic nutrient inputs on phytoplankton community structure and biological diversity exacerbated by indiscriminate sewage discharge and phosphorus loads into the tributary from surrounding towns and proximal mining industries, respectively. The study shows that cyanobacteria are the dominant phytoplankton species in XXB, however, trace metal supply could trigger a change in community structure from Cyanophyta—dominated to Chlorophyta-dominated system. The contemporaneous influx of N and P, as well as Si, has been implicated to severely disrupt the phytoplankton community structure and composition through competition and succession which eventually cause the cyanobacteria to dominate. Generally, the study demonstrates an inverse relationship between increasing nutrient loads and phytoplankton biodiversity. High nutrients concentrations largely decrease biodiversity (H < 1) allowing the fast-growing phytoplankton taxa to dominate and outcompete their slow-growing opportunists. In other words, high nutrient state results in low diversity/evenness but high dominance of a certain group and vice versa. Therefore, information on nutrient dynamics, biodiversity index and relative abundance of phytoplankton could be principal in evaluating the influence of human activities under changing climatic disturbances in freshwater ecosystems. Further, our study recommends that prudent legislation be enacted to substantially reduce nutrient budget especially N, P, and Si as this would effectively guarantee a lasting solution to phytoplankton expansion in Xiangxi Bay and related tributaries under similar anthropogenic stressors.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2020.125960.

References


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