



# Limiting nutrient and its use efficiency of phytoplankton in a shallow eutrophic lake, Lake Kasumigaura

Takehiko Fukushima · Bunkei Matsushita

Received: 18 September 2020 / Revised: 27 March 2021 / Accepted: 16 April 2021 / Published online: 14 May 2021  
© The Author(s), under exclusive licence to Springer Nature Switzerland AG 2021

**Abstract** In the management of lakes for a healthy environment, it is crucial to assess the limiting nutrient(s) and understand its use efficiency of the phytoplankton production. We hypothesized that dissolved inorganic phosphorus (DIP) and/or nitrogen concentrations (DIN) describe the state of nutrient limitations on phytoplankton production. Using the 38 years of monthly limnological data at eight stations in Lake Kasumigaura, we analyzed the relationships between the available nutrients for building phytoplankton biomass and chlorophyll a (Chla). Better correlations were obtained by subtracting the nutrients in tripton (non-living particulate matter) from the total P and N (available nutrients: TP' and TN'). We determined the thresholds for P and N limitation (DIP:  $0.01 \text{ mg l}^{-1}$ ; DIN:  $0.15 \text{ mg l}^{-1}$ ) using the slopes of regression, and we examined this assessment in one of

four limitation conditions (only P, only N, both, or none) for the respective times and stations. Close relationships between TP' and Chla for only the P limitation data and between TN' and Chla for only the N limitation data were observed. Multiple regression models confirmed that TP' and TN' predominantly determined Chla in only the P and N limitation conditions, respectively. Factors affecting nutrient use efficiency were also discussed.

**Keywords** Nutrient limitation · DIP & DIN method · Available nutrient · Nutrient use efficiency · Spatiotemporal change

## Introduction

Anthropogenic eutrophication is one of the greatest threats to aquatic ecosystems (Steffen et al., 2015). Nutrient limitation of lake pelagic phytoplankton communities has been the subject of numerous studies and a focal area of research for decades (e.g., Vollenweider & Kerekes, 1980). There has been an extended debate over whether nitrogen (N) or phosphorus (P) is the nutrient that ultimately determines productivity in lakes (e.g., Lewis & Wurtsbaugh, 2008; Schindler et al., 2008; Sterner, 2008).

In general, either of two methodologies is used when discussing the nutrient limitation of

---

Handling editor: Judit Padisák.

---

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s10750-021-04593-y>.

---

T. Fukushima (✉)  
Ibaraki Kasumigaura Environmental Science Center, 1853  
Okijyuku, Tsuchiura, Ibaraki 3000023, Japan  
e-mail: [fukushima.takehik.fu@u.tsukuba.ac.jp](mailto:fukushima.takehik.fu@u.tsukuba.ac.jp)

T. Fukushima · B. Matsushita  
Faculty of Life and Environmental Sciences, University of  
Tsukuba, 1-1-1 Tennoudai, Tsukuba,  
Ibaraki 3058572, Japan

phytoplankton communities (Stumm, 2003; Davies et al., 2010). The first assesses the immediate needs of the extant plankton community and the factors affecting its composition. For example, the plankton physiological status was estimated using the addition of various nutrients (e.g., Maberly et al., 2002; Kolzau et al., 2014; Andersen et al., 2020). Phosphorus deficiency was evaluated by determining the phosphate turnover time in a radiophosphate uptake bioassay (e.g., Davies et al., 2010; Vandergucht et al., 2013). However, these methods are laborious and time-consuming and cannot be used to investigate the past status of the targeted water region.

In contrast, the second methodology for the nutrient limitation of phytoplankton communities focuses on long-term constraints on the potential phytoplankton biomass. Early large-scale comparisons across temperate lakes revealed a broad relationship between the phytoplankton biomass, commonly expressed as the concentration of chlorophyll *a* (Chl*a*), and the concentration of P expressed as total phosphorus (TP) (e.g., Vollenweider & Kerekes, 1980). “Total nutrient limitation” is generally recognized to indicate the restrictions of the phytoplankton biomass related to the proportion of the nutrient in limited supply, and it was considered to apply to longer time scales (Rast & Thornton, 2005; Schindler et al., 2008; Sterner, 2008). In addition to a correlation analysis, a principal component analysis (Li et al., 2014) and a multiple regression analysis (Guo et al., 2018) have been used to determine the limiting nutrient(s).

Phytoplankton community models that have been calibrated with the field data provided hints for the evaluation of the limiting factors (e.g., Elliott & May, 2008). It is more difficult to identify and validate the limiting nutrient(s) at any given moment than across a long period of time, but seasonal changes in limiting nutrients are expected in many water regions (Maberly et al. 2020). Season-specific nutrient management could be practiced if the precise changes in limiting nutrients can be identified.

Considering that bioassay experiments are rather laborious, an assessment system that uses the concentrations of dissolved nutrients (e.g., dissolved inorganic nitrogen [DIN], soluble reactive phosphorus [SRP], dissolved inorganic phosphorus [DIP]) was proposed for use in combination with the results of bioassays. Based on their nutrient addition experiments with water samples of 30 small ponds in

Cumbrian, Wales, Scotland and Northern Ireland, Maberly et al. (2002) demonstrated that N limitation was more likely at sites where the DIN was  $< 6.5 \text{ mmol m}^{-3}$  ( $0.091 \text{ mg l}^{-1}$ ) and that P limitation was most likely where the DIN was  $> 13 \text{ mmol m}^{-3}$  and the DIN:TDP (total dissolved P) molar ratio was  $> 250$  (i.e.,  $\text{TDP} < 0.0016 \text{ mg l}^{-1}$ ). Similarly, Kolzau et al. (2014) uses samples from four German lakes and they reported that P limitation was observed only when the SRP was  $< 0.010 \text{ mg l}^{-1}$ , and that N limitation was present only when the DIN was  $< 0.1 \text{ mg l}^{-1}$ . They suggested the possibility of predicting the limitation status based on the ambient dissolved nutrient concentrations. However, the applicability of the method using DIP and DIN to assess the limiting nutrient(s) was not examined in reference to the relationship between the total nutrient amount and the phytoplankton biomass.

In some situations, stoichiometry indices (e.g., the N:P ratio) have been used to assess the limiting nutrient (e.g., Guildford & Hecky, 2000), but the trophic level of the targeted water region should be considered (Schelske et al., 1999). The water temperature was considered as another important controlling factor for phytoplankton production (e.g., Hakanson et al., 2007; Hennemann & Petrucio, 2016). The impact of a rise in the water temperature on lake environments and ecosystems is receiving a greater amount of attention in the context of climate change.

Recently, Chorus & Spijkerman (2020) attempted to review and resolve the debate about N (co-) limitation, the N:P ratios (stoichiometry) and the need for the reduction of the N-load in lake management by summarizing the Colin Reynolds’ messages for understanding how nutrient limitation works. The key parts of the messages are based on differentiation into three nutrient fractions as follows. (i) Dissolved inorganic nutrients limit the rates of nutrient uptake. (ii) The cellular nutrient content (cell quota) limits the cell division rate. (iii) The total phosphorus (TP) and total nitrogen (TN) limit the amount of phytoplankton biomass. Chorus and Spijkerman concluded the correctness of the Colin Reynolds’ messages in eutrophication control, and they described the possibility of light limitation under non-nutrient-limiting conditions (i.e., soluble P  $> 0.003\text{--}0.01 \text{ mg l}^{-1}$  and dissolved N  $> 0.1\text{--}0.13 \text{ mg l}^{-1}$ ).

Maberly et al. (2020) used four approaches to assess nutrient limitation and applied the approaches to 17 small ponds (generally shallow) in the North-West Midlands in the UK. The four approaches used (i) the seasonal nutrient minima (SRP < 0.010 mg l<sup>-1</sup> for P limitation; DIN < 0.1 mg l<sup>-1</sup> for N limitation); (ii) the nutrient ratio (DIN/SRP > 20 for P limitation; DIN/SRP < 10 for N limitation); (iii) the Chla to nutrient ratio (Chla/TP > 0.3 for P limitation; Chla/DIN > 0.042 for N limitation); and (iv) stoichiometric modeling (Metabolake), because these indicators reflected different aspects of nutrient availability and their conversion into biomass. An evaluation of light limitation was included in their stoichiometric modeling.

Lake Kasumigaura is a shallow polymictic eutrophic lake characterized by high tripton (nonliving particulate matter) concentration (Fukushima et al., 2019). Field measurements of reservoirs in Missouri, USA indicated that the phosphorus bound to tripton was not associated with phytoplankton (Yuan & Jones, 2020). It is therefore necessary to pay careful attention to the specific method used when describing the available nutrients for phytoplankton production (hereafter, referred to as available nutrients) in lakes such as Lake Kasumigaura. In addition, decadal changes in dominant phytoplankton species have been observed accompanied by relatively abrupt shifts and notable changes in seasonal patterns of the phytoplankton biomass (Fukushima & Arai, 2015). Because large seasonal, decadal, and spatial variations in N/P ratio are characteristics of Lake Kasumigaura, the limiting nutrient(s) should be considered when these variations are evaluated.

We thus conducted the present study to (i) determine the threshold values of DIP and/or DIN for the assessment of the limiting nutrient(s) in phytoplankton production in the shallow eutrophic lake, Lake Kasumigaura; (ii) determine the validity and applicability of this assessment method for nutrient limitation compared to the use of the N:P ratio; (iii) evaluate the impacts of other factors, e.g., the water temperature, the tripton concentration (as an indicator of light availability) on the nutrient limitation; and (iv) analyze the nutrient use efficiency (i.e., the phytoplankton volume divided by the available nutrient amount) with regard to decadal changes in the lake's water quality, dominant phytoplankton species, etc., using the 38 years of monthly limnological data at

eight stations in Lake Kasumigaura. We tested our hypothesis that is based on two of the Colin Reynolds' key messages; i.e., (i) the dissolved nutrient concentrations indicate the state of nutrient limitation, and (iii) the amount of phytoplankton is proportional to the limiting available nutrient(s) (Fig. 1; details below).

## Methods

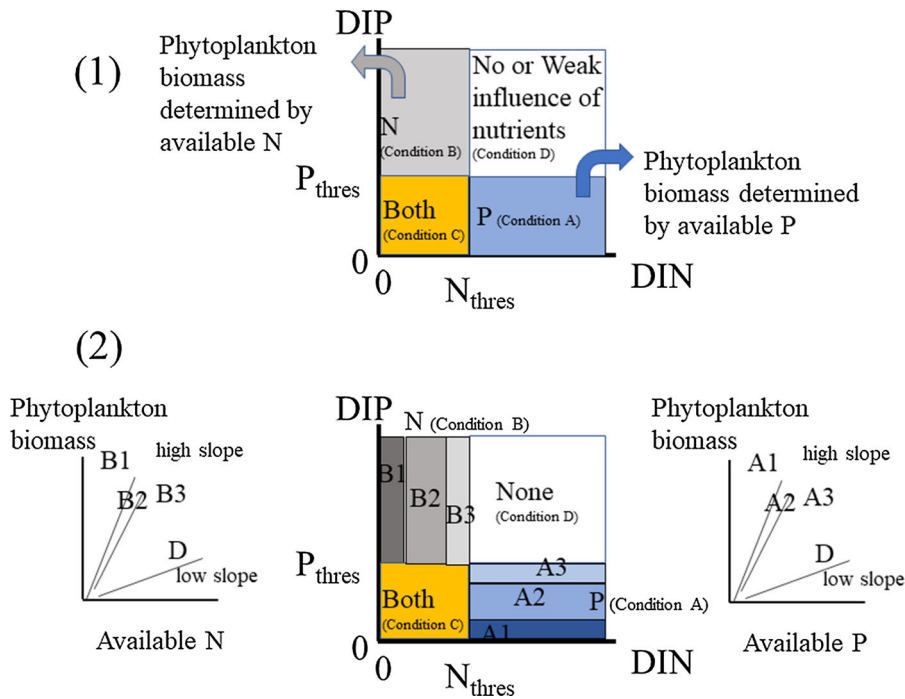
### Study site

Lake Kasumigaura is Japan's second largest lake (Lake Biwa is the largest), with a surface area of 171 km<sup>2</sup> (Fig. 2). The lake is characterized by high turbidity (yearly means of tripton: 3–25 mg dry weight of particulate matter [DWPM] l<sup>-1</sup>) and eutrophic conditions (TP: 0.084 mg l<sup>-1</sup>, TN: 1.01 mg l<sup>-1</sup>, Chla: 58.4 µg l<sup>-1</sup>, = the averages during 1979–2016 at Station [St.] 1) (Fukushima et al., 2019) due to its shallowness (mean depth of 3.4 m; max. depth of 7.0 m) and the intensive human activity (e.g., agriculture, stock raising, manufacturing industry) in the watersheds (WEPA, 2020). Its 1426-km<sup>2</sup> catchment area (excluding the lake area) is approx. 30% forest, 25% paddy fields, 25% ploughed fields, 10% residential and 10% other (Fukushima et al., 2007).

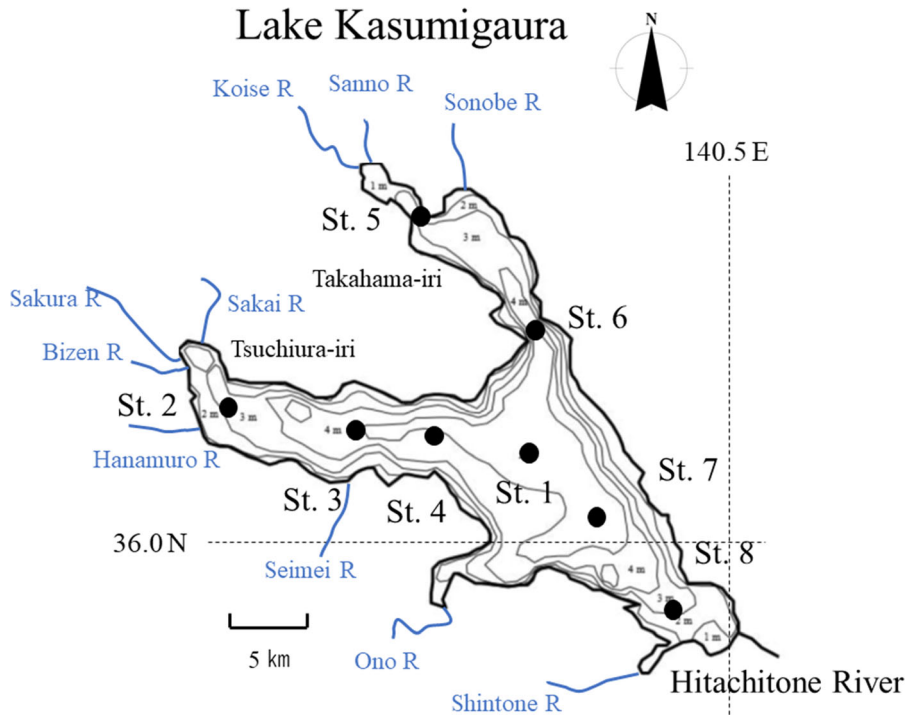
The apparent water residence time of Lake Kasumigaura is approx. 0.55 y. Diurnal stratification is sometimes formed under a weak-wind condition (Ishikawa & Tanaka, 1993), but vertical stratification is easily destroyed by strong winds due to the lake's shallow depth (Fukushima & Muraoka, 1981). The lake is thus classified as a warm polymictic lake. It has two large bays: Takahama-iri (bay) and Tsuchiura-iri. Water tends to flow through the lake from northwest to southeast, to the effluent Hitachitone River. Due to the lake's shallowness, processes related to water–sediment interactions such as sediment resuspension and the release of dissolved matter from sediments play a large role in the nutrient dynamics (Havens et al., 2001; Fukushima et al., 2010).

### Database and grouping

For the present analyses, we used the data on monthly water quality measured by the Kasumigaura River Office (KRO) of Japan's Ministry of Land,



**Fig. 1** Our hypothesis about the relationship between nutrients and phytoplankton biomass. (1) Conditions classified by the DIP and DIN concentrations, (2) The relationships between available nutrients and biomass in Condition A (only P limitation) and Condition B (only N limitation)



**Fig. 2** Lake Kasumigaura and sampling stations for water quality

Infrastructure and Transport (MLIT) collected at eight stations around the lake (Fig. 2) during the period 1979–2016 were used for the analysis. The KRO took water samples 0.5 m below the water surface. The annual means were calculated for the data from Jan to Dec. The data obtained at St. 1 (which is at the center of the lake) were used as the representative data of this lake because they roughly indicated the averages at all eight stations.

Of the 50 water quality parameters recorded in the KRO database, we focused on the following: TP, TN, dissolved inorganic phosphorus (DIP), DIN (sum of ammonium nitrogen [ $\text{NH}_4\text{-N}$ ], nitrite nitrogen [ $\text{NO}_2\text{-N}$ ] and nitrate nitrogen [ $\text{NO}_3\text{-N}$ ]), and Chla. We used the Chla data as the surrogate of phytoplankton biomass. Dissolved total phosphorus (DTP), dissolved organic phosphorus ( $\text{DOP} = \text{DTP} - \text{DIP}$ ), particulate phosphorus ( $\text{PP} = \text{TP} - \text{DTP}$ ), dissolved total nitrogen (DTN), dissolved organic nitrogen ( $\text{DON} = \text{DTN} - \text{DIN}$ ), particulate nitrogen ( $\text{PN} = \text{TN} - \text{DTN}$ ), suspended solids (SS), water temperature (WT), and other parameters were also used for the analyses.

These parameters were analyzed based on JIS K 0102-2016 testing methods. The tripton concentration was determined using the values of SS and Chla (i.e.,  $[\text{Trip}] = [\text{SS}] - \alpha[\text{Chla}]$ , where  $[\text{Trip}]$  is the tripton concentration [ $\text{mg l}^{-1}$ ],  $[\text{SS}]$  is the concentration of SS [ $\text{mgDWP m}^{-1}$ ],  $[\text{Chla}]$  is the concentration of Chla [ $\mu\text{g l}^{-1}$ ], and  $\alpha$  is the proportional coefficient 0.12; Fukushima et al., 2019). Phosphorus (tripP) and nitrogen (tripN) in tripton were calculated as SS multiplied by sediment P (1.3 mg g dry weight of sediments  $[\text{DWS}]^{-1}$ ) and N (8.5 mg g  $[\text{DWS}]^{-1}$ ) content, respectively (sediments of the surface 0–2 cm; Fukushima et al., 2010), but the applicability of this assumption is discussed below.

The outline for determining the concentrations of respective nutrient components was as follows. Water samples were separated into two subsamples: one was treated by filtration (glass fiber filter: pore size 1  $\mu\text{m}$ ) (referred to as the dissolved component) and the other subsample was unfiltered (referred to as the total). Potassium persulfate was used to oxidize all phosphorus compounds into phosphate under acidic conditions and potassium persulfate was used to oxidize all nitrogenous compounds into nitrate under alkaline condition. An ascorbic spectrophotometric method was then used to determine the DIP, DTP and TP (APHA et al. 1995). Spectrophotometric methods

were also used to determine  $\text{NH}_4\text{-N}$  (indophenol blue method),  $\text{NO}_2\text{-N}$  (naphthyl-diamine method) and  $\text{NO}_3\text{-N}$  (naphthyl-diamine method with cadmium reduction column) (APHA et al. 1995). These values were generally measured using a continuous flow instrument. The limits of detection were approx.  $0.001 \text{ mg l}^{-1}$  for phosphorus and  $0.004 \text{ mg l}^{-1}$  for nitrogen.

To analyze the influence of decadal changes on the relationship between nutrient and phytoplankton biomass, we separated the data into five periods (P1: 1979–1989; P2: 1990–1999; P3: 2000–2006; P4: 2007–2011; P5: 2012–2016) based on regime shift analyses (Fukushima & Arai, 2015; Fukushima et al., 2019; Kohzu et al., 2019). The decadal change in the tripton concentration was considered one of the main drivers of the regime shifts through the supply of silicon and light availability; this change was closely related to the changes in main ions and sediment properties (Fukushima et al. 2020). The characteristics of the respective periods were provided below in Supplementary Table 5 (1).

We analyzed the influence of phytoplankton groups on the relationship between nutrients and phytoplankton biomass in a similar manner. To determine the dominant phytoplankton group in Lake Kasumigaura (Supplementary Table 1), we quantified the phytoplankton as a biovolume ( $\mu\text{m}^3 \text{ ml}^{-1}$ ) at St. 1 using data provided by Japan's National Institute for Environmental Studies (NIES: Takamura & Nakagawa, 2012). Phytoplankton data at another site (between St. 5 and St. 6) were also available, but the characteristics of the changes were similar to those at St. 1. In a given month, the group with the largest recorded quantity was regarded as the dominant group among the following six groups: G1 (C–C, i.e., Cyanophyceae–Chroococcales), G2 (C–N, i.e., Cyanophyceae–Nostocales), G3 (C–O, i.e., Cyanophyceae–Oscillatoriales), G4 (B, i.e., Bacillariophyceae), G5 (Chl, i.e., Chlorophyceae), and G6 (others).

## Hypothesis and analysis

As illustrated in Fig. 1 (1), we hypothesized that the concentrations of DIP and/or DIN indicate the state of the nutrient limiting conditions on phytoplankton production; i.e., a P limitation is expected when the DIP is less than the P threshold ( $P_{\text{thres}}$ ); similarly, an N limitation is expected when the DIN is less than the N

threshold ( $N_{\text{thres}}$ ), based on the kinetics of phytoplankton growth and the ideas by Maberly et al. (2003) and Kolzau et al. (2014). We also hypothesized that there would be high correlations between the available P and Chla in Condition A (i.e., “only P limitation”) and between the available N and Chla in Condition B (“only N limitation”) as described below. We also expected that the slopes of the linear regression lines of Chla to available nutrients would be higher as the dissolved nutrient concentrations became smaller, as shown in Fig. 1 (2), because the phytoplankton volume (expressed by Chla) is nearly proportional to the nutrient amounts contained in phytoplankton (P, N, etc., respectively; the roughly available for phytoplankton production minus the dissolved nutrient).

Thus, significant differences in the slopes could help determine the values of  $P_{\text{thres}}$  and  $N_{\text{thres}}$ . If the hypothesis shown in Fig. 1 (1) is confirmed, we will be able to assess lake nutrient conditions using the DIP and DIN values (the ‘DIP & DIN method’), as ‘only P limitation’ in Condition A, ‘only N limitation’ in Condition B, ‘P & N limitation’ (or ‘both’) in Condition C, and no or weak influence of nutrients (hereafter, ‘no limitation’ or ‘none’) in Condition D. We performed a multiple regression analysis (dependent variable: Chla, independent variables: TP', TN' and WT; Brown et al., 2004) using the datasets in order to compare their influences on phytoplankton production. Tripton was added to the set of independent variables for the multiple regression analysis in Condition D (‘none’) to check the possibility of light limitation. The light attenuation coefficient in Lake Kasumigaura is strongly determined by the concentration of SS, with the contributions from phytoplankton and tripton (Yang et al., 2011; Fukushima et al., 2018). The analyses revealed that Chla correlated rather well with SS in each of the five periods (a self-shading effect was expected), but tripton varied widely among the periods; we thus used tripton in the multiple regression analysis to assess the influence of light on phytoplankton production.

Regarding available nutrients, we used TP and (TP–tripP) for P and TN, and we used (TN–tripN) and (TN–tripN–DON) for N, respectively, because nutrients contained in tripton (Schallenberg & Burns, 2004; Yuan & Jones, 2020) and/or DON (Kaushal & Lewis, 2005; Pisani et al., 2017) were considered less available for building phytoplankton biomass. We explain below why (TP–tripP) and (TN–tripN) were

chosen to represent the available P and N in this lake, respectively. Hereafter, (TP–tripP) and (TN–tripN) are expressed as TP' and TN', respectively.

We compared the applicability of the above DIP & DIN method with the use of the N/P ratio for Lake Kasumigaura. Moss et al. (2013) reported that a distribution TN/TP range of 3.6–13.5 (weight, molar ratio 8–30) was commonly used to separate a definite P limitation from a definite N limitation. Guilford & Hecky (2000) concluded that N-deficient growth was noticeable at TN/TP < 9 (weight, molar ratio 20), whereas P-deficiency growth consistency occurred when the TN/TP ratio was > 22.6 (weight, molar ratio 50), based on the nutrient and Chla dataset across a broad range of lakes and oceans. In the present study, we tested three thresholds for the TN'/TP' ratio (> 22.6, > 20, > 18) were tested for P limitation and three thresholds for the TN'/TP' ratio (< 7, < 8, < 9) for N limitation. A multiple regression analysis was also applied to the respective datasets, and the results were compared with those obtained by the new DIP & DIN method.

To elucidate the influences of the five periods, the stations, and the dominant phytoplankton groups on the relationship between Chla and nutrients, we compared the groups' ratios of Chla to nutrients (nutrient use efficiency; Chla/TP' in only P limitation and Chla/TN' in only N limitation) and the groups' multiple regression coefficients.

## Statistical methods

We determined the correlations and the differences between means (*t* test) and performed a regression analysis for significance at the level of  $P = 0.05$  (\*) or  $P = 0.01$  (\*\*) (Excel Statistics for 2016, BellCurve Social Survey Research Information Co., Tokyo and OriginPro 2017 J, OriginLab. Corp., Northampton, MA). A multiple regression analysis with the backward elimination technique (the threshold of  $P = 0.01$  was used for removing the independent variables; Excel Statistics for 2016) was also performed. All of the multiple regression coefficients presented herein were significant ( $P < 0.01$ ). The partial correlation coefficients of which variables remained through the backward elimination technique are provided in the tables (all significant  $P < 0.01$ ) to indicate how strongly each variable affected the Chla. Fitting to a polynomial equation was also used (OriginPro 2017J).

In order to calculate the percentages of variations, an analysis of variance (ANOVA) was conducted using R (R Core Team, 2020).

## Results

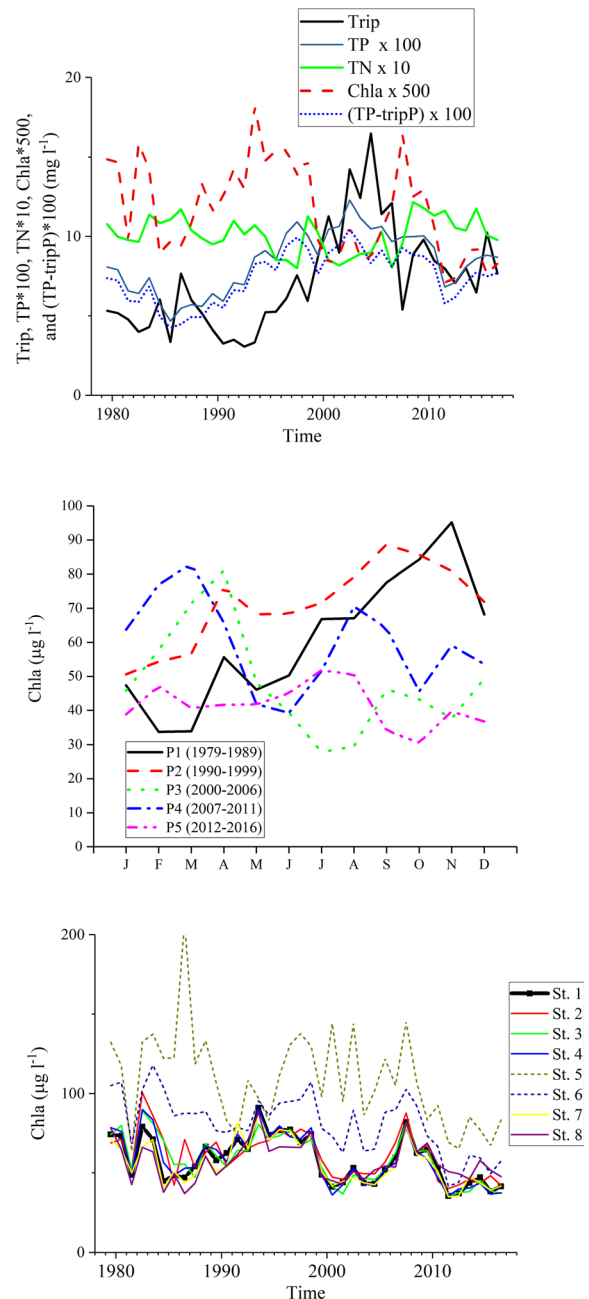
### Spatiotemporal changes in nutrient and phytoplankton concentrations

The annual averaged concentrations of TP and tripton at St. 1 were highly correlated ( $r = 0.68^{**}$  at St. 1, similarly at the other seven stations) and showed clear peaks in the first half of the 2000s (also  $r = 0.53^{**}$  between TP' and tripton; Fig. 3 (1)). In contrast, the annual averaged concentrations of Chla were inversely correlated with those of tripton ( $r = -0.52^{**}$ ) because the presence of tripton brought about a reduction of the light intensity (Nakamura & Aizaki, 2016). The decadal variation in TN was small and appeared random.

Annual peaks of Chla were observed in Nov (P1), Sep (P2), Apr (P3), Mar/Aug (P4) and Feb/Jul (P5) (Fig. 3 (2)) at St. 1. In contrast, TP (not shown), TP' (Supplementary Fig. 1 (1)) and DIP (Supplementary Fig. 1 (3)) showed peaks in summer (Jul–Sep) in almost all five periods, attributed to the release of P from the sediments by Fukushima et al. (2010). TN (not shown), TN' (Supplementary Fig. 1 (2)) and DIN (Supplementary Fig. 1 (4)) were higher in autumn and winter (Oct–Mar). The decadal and seasonal changes in the respective nutrient components at St. 1 are illustrated in Supplementary Fig. 2.

The annual peaks in DIP,  $\text{NH}_4\text{-N}$ , and  $\text{NO}_3\text{-N}$  were sharp and their heights varied between years. Periods of a quite low dissolved nutrient concentration were sometimes observed. The means at St. 1 were as follows; DIP, 0.009; DOP, 0.017; TripP, 0.009; (PP–TripP), 0.049, and TP, 0.084  $\text{mg l}^{-1}$ . Additional means at the station were  $\text{NH}_4\text{-N}$ , 0.046;  $\text{NO}_2\text{-N}$ , 0.009;  $\text{NO}_3\text{-N}$ , 0.178; DON, 0.420; TripN, 0.062; (PN–TripN), 0.295, and TN, 1.009  $\text{mg l}^{-1}$ .

Decadal and seasonal change patterns similar to those at St. 1 were observed at the other seven stations (Fig. 3 (3), Supplementary Fig. 3, etc.). Significant correlations among the stations were observed for all of the decadal TP, TN, and Chla changes with the exception of Chla at St. 5 ( $P < 0.05$  for the correlations between the yearly averaged values at any of two



**Fig. 3** Decadal, seasonal and sampling station differences in water quality items. (1) Yearly concentration changes in tripton, TP, TN, Chla and (TP–tripP) at St. 1, (2) Seasonal change in Chla in the respective periods at St. 1, (3) Decadal change in Chla at the eight stations

of the seven stations except St. 5). Almost all of the Chla seasonal changes at the eight stations showed significant correlations; the ratios satisfying  $P < 0.05$  were 25/28 in P1, 22/28 in P2, 25/28 in P3, and 24/28

in P4 (the total numbers of combinations [two of the eight stations] were 28). Unclear seasonal patterns (data not shown) were dominant in P5 (12/28;  $P < 0.05$ ).

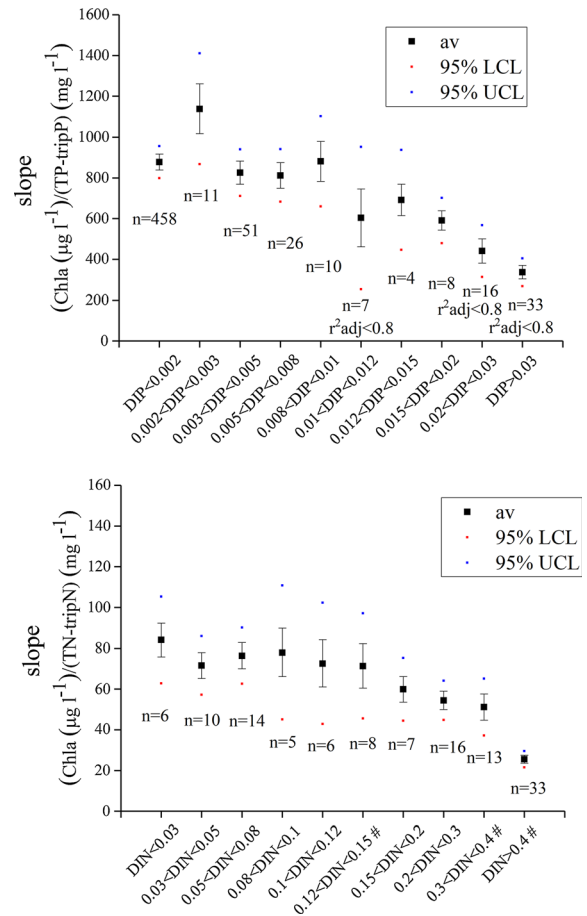
The percentages of variations in Chla, TP, and TN indicated that the temporal variations (expressed by “Year”, “Month”, “Year  $\times$  Month” in Supplementary Table 2) were large in Chla and TP, and that the stational variations in TN were relatively significant (“Station” in the table). These water-quality indicators were high near the stations close to large influent rivers (Sts. 2 and 5) and gradually decreased in the downstream directions (means of Chla: 58, 61, 59, 59, 108, 81, 56, and 57  $\mu\text{g l}^{-1}$  at St. 1 to St. 8, respectively). We next analyzed the relationship between nutrients and biomass as described below in Lake Kasumigaura where significant seasonal, decadal and stational variations in nutrients and biomass were observed.

Variations in the TN/TP ratio were low (weight basis  $< 10$ ) in the summer and high ( $> 20$ ) in Jan, Feb and Mar (Supplementary Fig. 4). Between 1995 and 2010, the TN/TP ratio was quite low in any month.

#### Estimation of $P_{\text{thres}}/N_{\text{thres}}$ and the relationship between Chla and available nutrients

For the analysis considering the hypothesis shown in Fig. 1 (2) (i.e., the phytoplankton biomass is nearly proportional to the amount of an available nutrient when the nutrient limits the production of phytoplankton), it was necessary to pre-set the thresholds. We thus began with the initial thresholds ( $P_{\text{thres}} = 0.010 \text{ mg l}^{-1}$ ,  $N_{\text{thres}} = 0.10 \text{ mg l}^{-1}$ ) based on previous bioassay experiments (Kolzau et al., 2014), and we revised the values to find the best combination of the thresholds.

We formed ten DIP groups (Fig. 4 (1)) and ten DIN groups (Fig. 4 (2)) for the respective settings. We then calculated the slope of regression and the adjusted  $r^2$  for the regression line with no intercept (dependent variable: Chla, independent variable:  $\text{TP}'$  [or  $\text{TN}'$ ]) for each group, because insignificant intercepts ( $P > 0.05$ ) were obtained for almost all of the regressions ( $\text{TP}'$ : 80%,  $\text{TN}'$ : 88%) and because Chla could be set at essentially zero when there was no available nutrient. The adjusted  $r^2$  values were usually  $> 0.8$  in the respective groups, and the slopes often decreased with higher DIP (or DIN) ranges (e.g.,

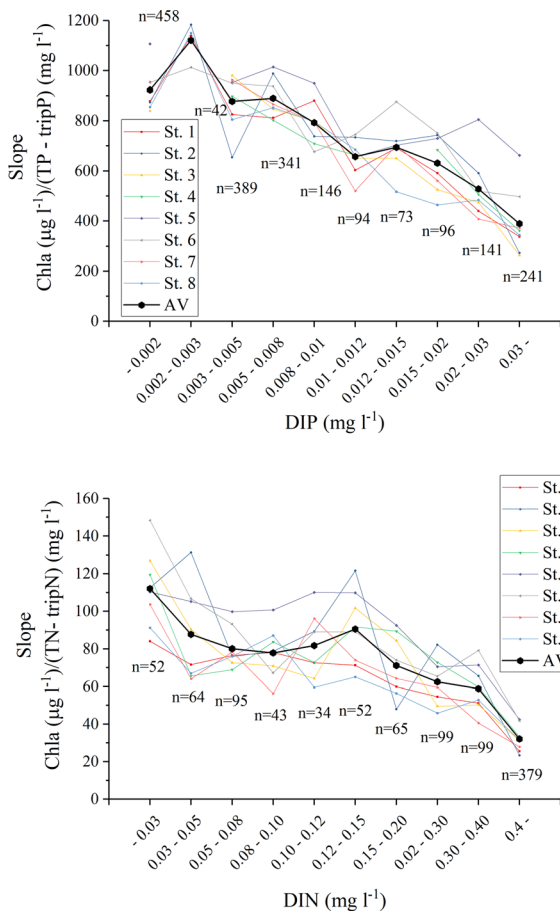


**Fig. 4** Slope of the regression of Chla to available nutrients with no intercept for the respective 10 ranges at St. 1. The mean, upper 95% confidence interval (CL), and lower 95% CL are shown.  $n$ : data number for the respective ranges; adjusted  $r^2 \geq 0.8$  (indicated when adjusted  $r^2$  is  $< 0.8$ ). (1) Chla to  $\text{TP}'$ , (2) Chla to  $\text{TN}'$

Fig. 4 for St. 1). By applying a t test for the means between two adjacent groups, we searched for significant differences between the groups to determine the threshold value.

The results revealed that the most pronounced differences were between the (0.008–0.010  $\text{mg l}^{-1}$ ) group and the (0.010–0.012  $\text{mg l}^{-1}$ ) group in the case of DIP ( $P < 0.01$ ) (Fig. 5 (1)) and between the (0.12–0.15  $\text{mg l}^{-1}$ ) group and the (0.15–0.20  $\text{mg l}^{-1}$ ) group in the case of DIN ( $P < 0.05$ ) (Fig. 5 (2)), thus indicating the thresholds for limitation ( $P_{\text{thres}}$ : 0.010  $\text{mg l}^{-1}$ ,  $N_{\text{thres}}$ : 0.15  $\text{mg l}^{-1}$ ) close to those proposed by Kolzau et al. (2014).





**Fig. 5** Slopes of the regression of Chla to available nutrients with no intercept for the respective 10 ranges at eight stations. Blanks indicate data number < 2. *n*: data number at all stations for the respective ranges. (1) Chla to TP', (2) Chla to TN'

The results of the multiple regression analyses applied to the data from all stations, differentiated for the four limitation conditions, showed high values for the adjusted regression coefficients up to 0.55 for both P limitation and for N limitation when using various combinations of the threshold values (Table 1 (1), (2)). The maximum adjusted *r*<sup>2</sup> values were observed to be close to the above-mentioned values of *P*<sub>thres</sub> and *N*<sub>thres</sub>, thus confirming the validity of the method described above for determining limitation thresholds. In Condition A (only P limitation), TN' and WT also showed significant partial correlations with Chla, but these partial correlation coefficients were rather lower than those for TP' (Table 1 (1)). Fairly high regression coefficients were obtained for the regression of Chla to TP' (Fig. 6 (1)). Here, *r*<sup>2</sup><sub>adjusted</sub> = 0.51, indicating that

more than half of the variations could be explained by TP'.

On the other hand, TN' was often the single variable determining Chla in Condition B (only N limitation) (Table 1 (2)). The high regression coefficients of Chla to TN' are also shown in Fig. 6 (2). These results confirm the hypothesis depicted in Fig. 1 (1) and (2), as do the lower adjusted multiple regression coefficients that we observed for the conditions of limitation by either both (Condition C) or neither of the two nutrients (Condition D). These lower coefficients may indicate the relatively smaller influences of nutrients and/or their non-linear influences.

In Condition D (None), tripton was included into the multiple regression analysis (*P*<sub>thres</sub>: 0.010 mg l<sup>-1</sup>, *N*<sub>thres</sub>: 0.15 mg l<sup>-1</sup>), but this parameter did not prove relevant when using all of the stations' data were used (partial correlation coefficient = - 0.17, *P* > 0.05). However, for a few individual sampling stations, the multiple regression analysis did reveal a significant negative influence of tripton, indicating the possible light limitation (partial correlation coefficient: - 0.40\*\* at St. 4, - 0.28\*\* at St. 5, - 0.32\*\* at St. 6, insignificant at the other stations). Tripton was also included in Conditions A (only P), B (only N) and C (Both), but the results were insignificant.

By the way, the correlation coefficient between TP' and Chla (*r* = 0.72) was higher than that between TP and Chla (*r* = 0.69) for the data in Condition A (only P). Similarly, the correlation coefficient between TN' and Chla (*r* = 0.74) was higher than that between TN and Chla (*r* = 0.72) and that between (TN'–DON) and Chla (*r* = 0.73) in Condition B (only N). This confirms that subtracting nutrient fractions bound to tripton gives a better predictor of biomass than the total concentration.

#### Estimation of nutrient limitation

Using the proposed threshold concentrations, we estimated the changes in limiting nutrient (e.g., St. 1: Table 2; St. 2: Supplementary Table 3). At St. 1, P was usually the limiting nutrient from Nov to May, whereas N was sometimes the limiting nutrient from Mar to Sep. Both nutrients probably affected the phytoplankton production from Mar to May. After the latter half of the 1990s, in general neither P nor N limited the phytoplankton amount from Jul to Oct. A similar tendency was observed at St. 2, but the

**Table 1** Results of the multiple regression analysis with the backward elimination technique on the data grouped into the four Conditions in Fig. 1

(1) Condition A (only P limitation)										
$r^2_{\text{adjus}}$ (number)		$P_{\text{thres}}$ (mg l <sup>-1</sup> )								
		0.005			0.01			0.02		
$N_{\text{thres}}$ (mg l <sup>-1</sup> )	0.10	0.54 (1016)			0.54 (1583)			0.47 (1897)		
	0.15	0.54 (889)			0.54 (1376)			0.47 (1639)		
	0.20	0.55 (783)			0.54 (1214)			0.47 (1450)		
Partial correlation coefficient with Chla (significant regression parameter $P < 0.01$ )		$P_{\text{thres}}$ (mg l <sup>-1</sup> )								
		0.005			0.01			0.02		
		TP'	TN'	WT	TP'	TN'	WT	TP'	TN'	WT
$N_{\text{thres}}$ (mg l <sup>-1</sup> )	0.10	0.64	0.16	0.19	0.65	0.16	0.16	0.62	0.12	0.08
	0.15	0.63	0.17	0.19	0.64	0.15	0.16	0.61	0.11	0.07
	0.20	0.63	0.18	0.18	0.64	0.17	0.16	0.60	0.13	0.08
(2) Condition B (only N limitation)										
$r^2_{\text{adjus}}$ (number)		$P_{\text{thres}}$ (mg l <sup>-1</sup> )								
		0.005			0.01			0.02		
$N_{\text{thres}}$ (mg l <sup>-1</sup> )	0.10	0.50 (557)			0.55 (254)			0.48 (114)		
	0.15	0.52 (723)			0.55 (340)			0.50 (149)		
	0.20	0.51 (844)			0.55 (405)			0.51 (187)		
Partial correlation coefficient with Chla (significant regression parameter $P < 0.01$ )		$P_{\text{thres}}$ (mg l <sup>-1</sup> )								
		0.005			0.01			0.02		
		TP'	TN'	WT	TP'	TN'	WT	TP'	TN'	WT
$N_{\text{thres}}$ (mg l <sup>-1</sup> )	0.10	0.22	0.61			0.74			0.70	
	0.15	0.25	0.61	- 0.11		0.74			0.71	
	0.20	0.27	0.61	- 0.13	0.14	0.66		0.20	0.54	
(3) Condition C (P and N limitation)										
$r^2_{\text{adjus}}$ (number)		$P_{\text{thres}}$ (mg l <sup>-1</sup> )								
		0.005			0.01			0.02		
$N_{\text{thres}}$ (mg l <sup>-1</sup> )	0.10	0.36 (781)			0.39 (1084)			0.40 (1224)		
	0.15	0.38 (908)			0.43 (1291)			0.43 (1482)		
	0.20	0.39 (1014)			0.44 (1453)			0.44 (1671)		
Partial correlation coefficient with Chla (significant regression parameter $P < 0.01$ )		$P_{\text{thres}}$ (mg l <sup>-1</sup> )								
		0.005			0.01			0.02		
		TP'	TN'	WT	TP'	TN'	WT	TP'	TN'	WT
$N_{\text{thres}}$ (mg l <sup>-1</sup> )	0.10	0.38	0.37	- 0.10	0.41	0.41	- 0.10	0.31	0.52	
	0.15	0.41	0.36		0.45	0.42	- 0.08	0.35	0.52	
	0.20	0.42	0.36		0.47	0.42		0.37	0.51	

**Table 1** continued

(4) Condition D (no limitation)										
$r^2_{\text{adjus}}$ (number)		$P_{\text{thres}}$ (mg l <sup>-1</sup> )								
		0.005			0.01			0.02		
$N_{\text{thres}}$ (mg l <sup>-1</sup> )	0.10	0.35 (1291)			0.38 (727)			0.50 (413)		
	0.15	0.36 (1128)			0.40 (641)			0.52 (378)		
	0.20	0.37 (1007)			0.41 (576)			0.53 (340)		
Partial correlation coefficient with Chla (significant regression parameter $P < 0.01$ )		$P_{\text{thres}}$ (mg l <sup>-1</sup> )								
		0.005			0.01			0.02		
		TP'	TN'	WT	TP'	TN'	WT	TP'	TN'	WT
$N_{\text{thres}}$ (mg l <sup>-1</sup> )	0.10	0.53	0.18	-0.11	0.57	0.23	0.61	0.33		
	0.15	0.53	0.20	-0.14	0.58	0.26	0.62	0.36		
	0.20	0.53	0.23	-0.13	0.58	0.28	0.61	0.37		

Adjusted multiple regression coefficient  $r^2$  (data number) and partial correlation coefficients with Chla (only when the variable remained significant [ $P < 0.01$ ] in the multiple regression analysis) for the different combinations of  $P_{\text{thres}}$  and  $N_{\text{thres}}$ . Dependent variable: Chla, Independent variables: TP', TN', WT. Details are provided in the text

percentage of N limitation (only N and Both in the tables) was rather small compared to that at St. 1. Generally, the percentage of N limitation was low and that of no nutrient limitation (None) was high near the influent rivers (Sts. 2 and 5). The percentage of both P and N limitation (Both) increased downstream close to Lake Kasumigaura's outflow river (Supplementary Fig. 5).

#### Comparison with the N/P ratio method

With the N/P ratio method, rather lower adjusted regression coefficients ( $< 0.35$ ) and rather lower partial correlation coefficients ( $< 0.53$ ) were obtained (Table 3), indicating the superiority of the DIP & DIN method for assessing the limiting nutrient. In addition, the number of P limitation by N/P ratio method ( $< 1000$ ) was smaller than that provided by the DIP & DIN method (1,376 for the standard threshold). The estimated limiting nutrient indicated by the N/P ratio method revealed the dominance of a decadal trend over the seasonal pattern (Supplementary Table 4).

Differences in the relationships between Chla and nutrients in the periods and dominant phytoplankton groups

High  $r^2_{\text{adjust}}$  values were observed in some groups according to both period and dominant phytoplankton

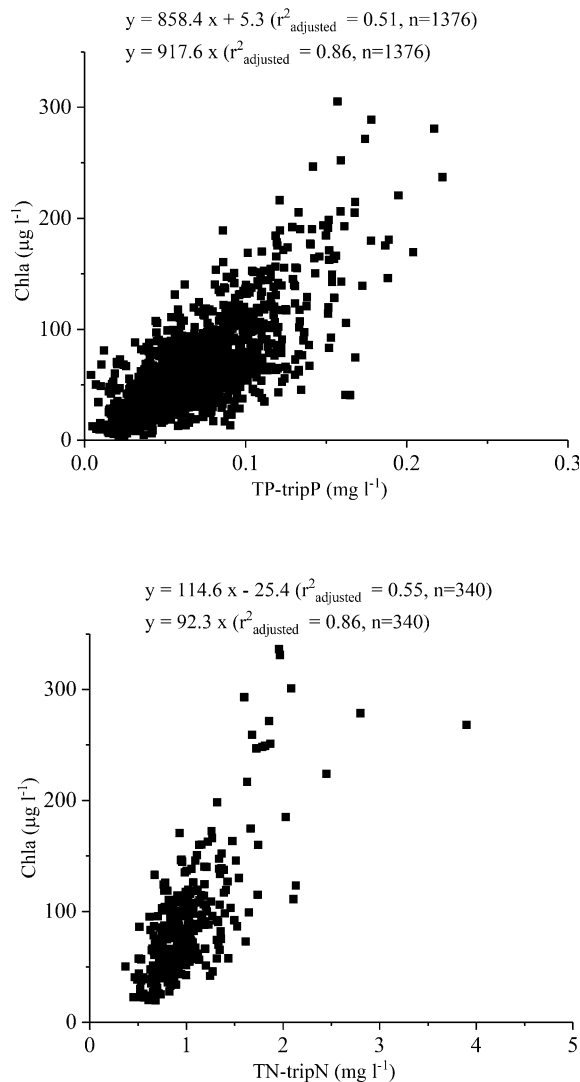
(Supplementary Table 5; e.g., Periods 2 and 4 in Condition A [only P limitation]), indicating that Chla was linearly correlated with nutrients in these groups. Particularly in most of the combinations of period groups, the means of Chla/nutrient ratios were significantly different, suggesting that these relationships differed by period groups. Higher ratios of Chla to nutrients were observed in Periods 1 and 2. Compared to the grouping by period, the grouping by dominant phytoplankton gave smaller significant differences between the groups, particularly in Condition A (only P limitation). Relatively smaller proportions of significant differences between the stations and lower  $r^2_{\text{adjust}}$  values in these groups indicated that the station differences in the relationship between Chla and nutrients were not large.

## Discussion

Validity of the estimation method for nutrient limitation

#### *Available nutrient for phytoplankton growth*

The correlation coefficient between TP' (or TN') and Chla was higher than that between TP (or TN) and Chla. In shallow lakes where sediment resuspension often occurs (Seki et al., 2006; Fukushima et al.,



**Fig. 6** Relationships between available nutrients and Chla for the entire study period at the eight stations. (1) TP' vs. Chla in Condition A (only P limitation), (2) TN' vs. Chla in Condition B (only N limitation)

2019), lake water contains resuspended particulate nutrients. Because these nutrients must be decomposed before uptake by phytoplankton, an experimental study with bioassays (Schallenberg & Burns, 2004) indicated that the amount of phytoplankton-available nutrients entrained from resuspended sediments was small. Much of the P bound to allochthonous suspended matter and/or resuspended sediments is considered to be not biologically available (Reynolds & Davies, 2001; Yuan & Jones, 2020).

In this study, we used the P and N contents of surface sediments (0–2 cm). It is possible that such contents can change. When we used the doubled concentration of tripton P ( $2.6 \text{ mg g}^{-1}$ ) for the calculation ( $\text{TP}'' = \text{TP} - \text{triptonP}' = \text{TP} - \text{triptonP}' \times 2$ ), a higher correlation coefficient was obtained between  $\text{TP}''$  and Chla ( $r = 0.66^{**}$  compared to  $r = 0.63^{**}$  between  $\text{TP}'$  and Chla at St. 1), but quite low or negative values of  $\text{TP}''$  were sometimes observed. More accurate measurements of the P and N contents of tripton are needed to determine the precise relationships between phytoplankton-available nutrients and Chla.

In the case of N, we also checked ( $\text{TN}' - \text{DON}$ ), but a smaller correlation coefficient was obtained. Goma (1995) reported that the N proportions of urea and dissolved-free amino acid (which are likely to be involved in phytoplankton production) were  $\sim 10\%$  in DON in Lake Kasumigaura. Other fractions in DON would also be not very refractory.

#### *The plankton chemical composition and linearity between Chla and available nutrient*

The ratio of Chla to TP under the P limitation condition was  $921 \text{ } \mu\text{gChla mgTP}^{-1}$  (averaged from a DIP range of  $< 0.002 \text{ mg l}^{-1}$  to that of  $0.008\text{--}0.01 \text{ mg l}^{-1}$  in Fig. 5 (1)). Similarly, the ratio of Chla to TN under the N limitation was  $88.3 \text{ } \mu\text{gChla mgTN}^{-1}$  (Fig. 5 (2)). The N to P ratio (weight) was estimated as 10.4 using the above ratios. Maberly et al. (2020) used a ratio of  $> 300 \text{ } \mu\text{gChla mgTP}^{-1}$  for P limitation and a ratio of  $> 42 \text{ } \mu\text{gChla mgTN}^{-1}$  for N limitation to count the number of nutrient-limited months. Our obtained ratios of Chla to TP or TN were larger than these respective values, indicating the nutrient limitation state.

Rios et al. (1998) reported the elemental and biochemical composition of phytoplankton based on the particulate matter samples taken in an estuarine area. The Chla to P ratio and the Chla to N ratio for diatoms were 1000 and  $166 \text{ } (\mu\text{g mg}^{-1})$ , respectively, and our obtained ratios were close to these values. Because field samples are sometimes taken under a non-nutrient limitation, these ratios of such samples may be smaller than those under nutrient limitation due to luxury uptake (Roelke et al., 1999). However, we did not observe this type of tendency in the present investigation. Regarding to the N to P weight ratio of

**Table 2** Estimated limiting nutrient at St. 1 (DIP & DIN method)

	J	F	M	A	M	J	J	A	S	O	N	D
1979	2	3	2	2	2	3	3	3	3	1	1	1
1980	1	0	0	1	1	2	2	2	3	3	3	3
1981	1	1	1	1	3	3	3	3	2	3	3	3
1982	3	1	1	3	3	3	1	3	3	1	1	3
1983	3	1	1	2	2	3	3	3	0	1	1	1
1984	3	1	1	1	1	3	2	2	2	2	1	1
1985	1	1	1	3	3	3	1	0	3	1	1	1
1986	1	1	1	1	3	3	3	2	2	0	1	1
1987	1	1	1	3	0	3	3	2	1	3	3	3
1988	1	1	1	2	3	3	3	3	0	1	1	1
1989	1	1	1	1	1	2	1	0	2	0	1	1
1990	1	1	1	1	1	3	3	3	3	3	1	1
1991	1	1	1	3	3	3	1	0	2	1	1	1
1992	1	1	1	1	3	1	3	0	2	3	3	3
1993	1	1	1	3	3	3	3	3	0	1	1	3
1994	1	1	1	3	3	3	2	2	2	1	3	3
1995	3	3	3	3	3	3	3	3	3	3	3	3
1996	3	1	1	3	3	3	3	2	2	3	3	3
1997	3	2	3	3	3	3	2	0	2	3	1	3
1998	3	3	3	3	3	3	2	2	0	0	0	1
1999	1	1	0	3	3	3	0	0	0	2	3	3
2000	3	3	3	3	3	3	3	0	0	0	0	1
2001	1	1	1	3	3	3	2	0	3	3	3	3
2002	3	1	3	3	3	3	3	0	0	0	0	0
2003	0	1	1	3	3	3	2	0	0	3	2	1
2004	1	1	0	3	3	3	0	0	2	0	1	1
2005	1	1	1	3	3	3	0	0	0	0	0	1
2006	3	1	3	3	3	3	2	3	3	2	3	3
2007	3	3	3	3	3	3	0	3	3	2	3	3
2008	3	3	3	3	3	3	1	1	0	0	1	3
2009	1	1	3	3	2	3	0	3	2	0	1	3
2010	3	3	3	3	3	3	0	2	0	0	1	1
2011	1	1	1	3	3	3	1	3	3	0	1	1
2012	1	1	1	1	0	0	1	2	0	0	3	1
2013	3	3	1	3	2	3	3	3	0	0	0	1
2014	1	1	1	1	3	3	2	0	0	0	1	1
2015	1	1	1	1	3	2	0	2	0	0	0	1
2016	1	3	3	3	3	3	2	0	0	0	1	1

0	1	2	3
None	Only P	Only N	Both

oceanic phytoplankton, the values of 7.2 (weight, molar ratio: 16) or 9 (weight, molar ratio: 20) were often assumed (Goldman et al., 1979), and these are similar to our N to P ratio.

We next examined the linearity of the relationship between Chla and available nutrients, i.e., the constancy of nutrient use efficiency over wide nutrient ranges. Polynomial equations (second-order with no intercept) were fitted to the results of Fig. 6. Although

**Table 3** Results of the multiple regression analysis with the backward elimination technique using the data grouped by N/P ratio

(1) P limitation											
$r^2_{\text{adjus}}$ (number)	TN/TP										
	> 22.6			> 20			> 18				
	0.35 (640)			0.31 (814)			0.32 (997)				
Partial correlation coefficient (significant regression $P < 0.01$ )	TN/TP										
	> 22.6			> 20			> 18				
	TP'	TN'	WT	TP'	TN'	WT	TP'	TN'	WT		
	0.47	-0.14	0.18	0.45	-0.10	0.10	0.47	-0.09	0.09		
(2) N limitation											
$r^2_{\text{adjus}}$ (number)	TN/TP										
	< 7			< 8			< 9				
	0.28 (263)			0.33 (497)			0.35 (773)				
Partial correlation coefficient (significant regression $P < 0.01$ )	TN/TP										
	< 7			< 8			< 9				
	TP'	TN'	WT	TP'	TN'	WT	TP'	TN'	WT		
				0.53	-0.21	0.14	0.41	-0.22	0.16	0.41	-0.19

Expressions similar to Table 1

the coefficients of non-linear terms were significant, the results indicated that the influence of non-linearity was negligible in the ranges up to  $0.2 \text{ mg l}^{-1}$  for TP and  $2 \text{ mg l}^{-1}$  for TN, respectively (Supplementary Fig. 6 (1) and (2)).

#### Determination of threshold values

We statistically determined the threshold values ( $P_{\text{thres}}$  and  $N_{\text{thres}}$ ), using the slopes of the regression lines (Fig. 5). Although a multiple regression analysis (Table 1) is probably helpful for determining these values, the procedure can be rather vague. It is difficult to obtain a definite separation based on the thresholds for data that are close to these values, and it is apparent that the assessment of nutrient limitation by the method that we propose herein roughly indicates the tendency of the nutrient state.

Comparison of the DIP & DIN method with previous methods

#### A bioassay experiment and an ecological model

Maberly et al. (2002) conducted a nutrient addition experiment using samples taken in 30 small ponds (TP:  $0.005\text{--}0.047 \text{ mg l}^{-1}$ , DTN:  $0.12\text{--}0.50 \text{ mg l}^{-1}$ , and Chla:  $0.6\text{--}10 \mu\text{g l}^{-1}$ ) from Apr to Aug, and they reported that N limitation occurred under the condition of  $\text{DIN} < 0.091 \text{ mg l}^{-1}$  and  $\text{DIN/DTP}$  weight ratio  $< 23.9$ . Based on a similar experiment using samples taken from four German lakes (TP:  $0.026\text{--}0.103 \text{ mg l}^{-1}$ , TN:  $0.59\text{--}1.49 \text{ mg l}^{-1}$ , and Chla:  $15\text{--}61 \mu\text{g l}^{-1}$ ) during the months from Mar to Sep, Kolzau et al. (2014) observed  $\text{DIN} < 0.10 \text{ mg l}^{-1}$  for N limitation. These DIN threshold concentrations were lower than our results ( $0.15 \text{ mg l}^{-1}$ ). Regarding P limitation, the threshold of  $\text{TDP} < 0.0016 \text{ mg l}^{-1}$  (Maberly et al., 2002) was lower and the threshold of  $\text{DIP} < 0.010 \text{ mg l}^{-1}$  (Kolzau et al., 2014) was the same as our DIP threshold ( $0.010 \text{ mg l}^{-1}$ ).

Chorus & Spijkerman (2020) reported the ranges below  $0.003\text{--}0.01\text{ mg l}^{-1}$  SRP or  $0.1\text{--}0.13\text{ mg l}^{-1}$  DIN for the limitation of uptake rates, and Maberly et al. (2020) used  $\text{SRP} < 0.01\text{ mg l}^{-1}$ , and  $\text{DIN} < 0.10\text{ mg l}^{-1}$  to assess the limitation in the respective months. Xiao et al. (2018) reported that the Michaelis constant ( $K_m$ ) of extracellular aminopeptidase was positively related to the ambient ammonium concentration, suggesting that nutrient limitation occurs up to a higher nutrient concentration as the ambient concentration increases. It could be that high P and N thresholds were observed in eutrophic Lake Kasumigaura. Regarding P, Fastner et al. (2016) reported that thresholds inducing a switch from cyanobacteria to other phytoplankton tend to be lower for lakes with a deeply mixed epilimnion compared to those for shallow lakes, implying differences in P uptake among the lakes.

Matsuoka et al. (1986) built an ecological model describing nutrient dynamics in lake Kasumigaura that was composed of 14 compartments. Two phytoplankton groups (Cyanobacteria and others) were simulated and the spatial and seasonal changes were validated. The half-saturation coefficients for their nutrient uptake submodel were set at  $0.0025\text{ mgP l}^{-1}$  and  $0.10\text{ mgN l}^{-1}$  for Cyanobacteria and  $0.0083\text{ mgP l}^{-1}$  and  $0.05\text{ mgN l}^{-1}$  for other phytoplankton. These values were similar to our nutrient thresholds. Similarly, the half-saturation coefficients of phytoplankton growth models applied to various lakes in the world ranged from  $0.001$  to  $0.05\text{ mg l}^{-1}$  for P and from  $0.025$  to  $0.4\text{ mg l}^{-1}$  for N (Somiya et al. 1989), indicating the nearly same order of magnitude as our thresholds.

#### *The TN/TP method*

The seasonal pattern of the N/P ratio that we observed in Lake Kasumigaura, i.e., low in summer and high in Jan, Feb and Mar, was similar to the patterns observed in Lake Taihu (Xu et al., 2010), many Danish lakes (Sondergaard et al., 2017), and four Nepalese lakes (Rowland et al., 2019). N limitation in summer and P limitation in other seasons were suggested by their nutrient addition assays.

As shown in Supplementary Table 4 based on the N/P method, P limitation usually occurred in autumn and winter during the periods 1979–1993 and after 2010 at St. 1. In contrast, N limitation occurred mainly in spring and summer during the period 1992–2007.

These tendencies generally agreed with those in Table 2, but the details differed. At St. 1, 53 of the 144 months of only P limitation revealed by the DIP & DIN method satisfied the condition of  $\text{TN}/\text{TP}' > 22.6$  (64 for  $\text{TN}/\text{TP}' > 20$ , 78 for  $\text{TN}/\text{TP}' > 18$ ), and 16 out of the 49 months of only N limitation shown by the DIP & DIN method satisfied the condition of  $\text{TN}/\text{TP}' < 9$ . In contrast, 53 of the 66 months when  $\text{TN}/\text{TP}'$  was  $> 22.6$  (16/90 in the case of N) were classified as only P limitation by the DIP & DIN method. The agreement between these two methods was better for P than for N. Finally, the inferiority of the TN/TP method compared to the DIP & DIN method for assessing the nutrient limitation condition was partly because some data belong to Conditions C (Both) or D (None) were classified into the P or N limitation groups.

Factors affecting the relationship between nutrients and phytoplankton biomass

#### *Light limitation*

As shown in Fig. 3 (1), the annually averaged Chla values were inversely correlated with tripton, suggesting light limitation during the high-tripton period (P3). However, the multiple regression analysis did not show a clear influence of tripton on monthly Chla in Condition D (None). While tripton responds quickly to wind speed by the wind wave height (Seki et al., 2006), the variation in Chla proceeds rather slowly together with those in TP and TN. It would thus be substantially difficult for such statistical analyses to determine whether light limitation occurred or not. In addition, analyses at the respective stations would be reasonable because the degree of light influence on phytoplankton production depends on the water depth of the sampling station.

#### *Differences due to periods and dominant phytoplankton and the influence of water temperature*

The ratios of Chla to nutrients were significantly different between several combinations of P1–P5 (Supplementary Table 5), suggesting changes in the relationship among periods. In contrast, the relationships did not significantly change according to dominant phytoplankton groups. As explained above, several items related to water quality, (e.g., tripton,

light availability, and dominant phytoplankton group) changed largely from one period to another. It is possible that the errors in the estimation of the available nutrient amount were produced due to an over- or underestimation of the tripton concentration and the nutrient content in tripton. However, it was difficult to ascertain the key factor that determine the nutrient use efficiency ( $\text{Chla}/\text{TP}'$ ,  $\text{Chla}/\text{TN}'$ ).

In addition, the influence of water temperature (WT) on the prediction of Chla was not very large, because the partial correlation coefficients with Chla were small and sometimes insignificant (Table 1). This finding indicates that a quasi-steady state between nutrients and Chla was attained at almost all timepoints and stations. The not very large influence of WT on phytoplankton production is similar to the influence in tropical lakes, where the maximum phytoplankton biomass is often observed during the winter (Wetzel, 2001).

### *Stational difference*

The percentages of both P and N limitation were high at Sts. 1, 7 and 8, which were located far from the main inflow rivers of Lake Kasumigaura (Supplementary Fig. 5). This longitudinal change was a result of the gradual decrease in nutrient concentrations due to sedimentation of the produced phytoplankton biomass (Supplementary Fig. 1 (3), Supplementary Fig. 3). Our finding that the differences among sampling stations in the relationship between Chla and nutrients were not very large indicated that the nutrients predominantly affected the Chla production in the entire lake.

### **Conclusions**

Although the determination of limiting nutrient(s) is worthwhile for lake management, the existing bioassays have been laborious and time-consuming. We tested the hypothesis illustrated in Fig. 1 and attempted to develop a new novel method to assess the limiting nutrient(s) using DIP and DIN concentrations at any given moment (DIP & DIN method). We examined the combination of two ideas regarding nutrient limitation (uptake rates expressed by dissolved nutrients and the carrying capacity by total available nutrients), and the results of our analyses

validated the DIP & DIN method as useful. When the relationship between Chla and total available nutrients was analyzed, the conditioning based on the dissolved nutrient concentration (grouping by combinations) was more meaningful and credible for determining the limiting nutrient compared to the conventional analysis without conditioning. In addition, the idea of nutrient use efficiency can be used for the management of nutrients in lakes and other waters. Although the key factors for determining this efficiency were not clearly elucidated in this study, further investigations can be expected to be informative.

In shallow lakes where sediment resuspension frequently occurs, the correction of the tripton component is important for evaluating the available nutrient concentration. Our hypothesis was confirmed; the scheme classifying the four nutrient limitation states (only P, only N, both, or none) was applied to 38 years of monthly limnological data observed at eight stations in the shallow eutrophic Lake Kasumigaura, and this method showed better performance compared to the N/P method (which has been applied to many water regions). We observed decadal, seasonal and sampling station differences in the relationship between available nutrients and Chla, i.e., information on the limiting nutrient, indicating that the DIP & DIN method could be a useful tool for understanding the dynamics of nutrients and phytoplankton and for better nutrient management. In Lake Kasumigaura, the influence of the water temperature on phytoplankton production is not very large. We also expected the possibility of light limitation in Condition D (no or weak influence of nutrients), but a multiple regression analysis did not indicate this. A new parameter indicating the degree of light limitation in a water column on a timescale of phytoplankton variation is necessary to evaluate this limitation correctly.

To validate the DIP & DIN method, cross-checking with a bioassay should be done in some lakes. Application trials to other water regions and/or periods are expected and necessary. Because a large dataset is needed to determine the threshold concentrations even in a lake, the accumulation of such information in various types of lakes and the integration of findings among the lakes are crucial for the further use of the DIP & DIN method.



**Acknowledgements** This work was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sport, Science and Technology (MEXT), Japan (17H04475 and 17H01850). The data on lake water and sediment quality were provided by the Kasumigaura River Office, Kanto Regional Development Bureau, Ministry of Land, Infrastructure, Transport and Tourism (MLIT). Monitoring data on water quality and phytoplankton were also provided by the National Institute for the Environmental Studies (NIES). We are grateful to Dr. Yumi Magahama and Dr. Mariko Furukawa for helpful advice on the statistical analysis using R. We thank two anonymous reviewers for their valuable comments and suggestions for improving the quality of the manuscript.

**Author contributions** TF designed this study and analyzed the data. BM commented on draft version of the article. All authors have approved the final article.

## Declarations

**Conflicts of interest** There is no conflict of interest.

## References

- Andersen, I. M., T. J. Williamson, M. J. Gonzalez & M. J. Vanni, 2020. Nitrate, ammonium, and phosphorus drive seasonal nutrient limitation of chlorophytes, cyanobacteria, and diatoms in a hyper-eutrophic reservoir. *Limnology Oceanography* 65(5): 962–978.
- APHA, AWWA, WEF, 1995. Standard Methods for the Examination of Water and Wastewater, 19th ed. APHA, Washington.
- Brown, J. H., J. F. Gillooly, A. P. Allen, V. M. Savage & G. B. West, 2004. Towards a metabolic theory in ecology. *Ecology* 85(7): 1771–1789.
- Chorus, I. & E. Spijkerman, 2020. What Colin Reynolds could tell us about nutrient limitation, N:P ratios and eutrophication control. *Hydrobiologia*. <https://doi.org/10.1007/s10750-020-04377-w>.
- Davies, J. M., W. H. Nowlin, B. Matthews & A. Mazumder, 2010. Temporal discontinuity of nutrient limitation in plankton communities. *Aquatic Sciences* 72(4): 393–402.
- Elliott, J. A. & L. May, 2008. The sensitivity of phytoplankton in Loch Leven (UK) to changes in nutrient load and water temperature. *Freshwater Biology* 53(1): 32–41.
- Fastner, J., S. Abella, A. Litt, G. Morabito, L. Voros, K. Palfy, D. Straile, R. Kiimmerlin, D. Matthews, M. G. Phillips & I. Chorus, 2016. Combating cyanobacterial proliferation by avoiding or treating inflows with high P load-experiences from eight case studies. *Aquatic Ecology* 50(3): 367–383.
- Fukushima, T. & H. Arai, 2015. Regime shifts observed in Lake Kasumigaura, a large shallow lake: analysis of a 40-year limnological record. *Lakes and Reservoirs: Research and Management* 20: 54–68.
- Fukushima, T. & K. Muraoka, 1981. Current and vertical mixing in a shallow lake. *Verhandlungen des Internationalen Verein Limnologie* 21: 141–149.
- Fukushima, T., M. Takahashi, B. Matsushita & Y. Okanishi, 2007. Land use/cover change and its drivers: a case in the watershed of Lake Kasumigaura, Japan. *Landscape and Ecological Engineering* 3: 21–31.
- Fukushima, T., K. Kamiya, Y. Onda, A. Imai & K. Matsushige, 2010. Long-term changes in lake sediments and their influences on lake water quality in Japanese shallow lakes. *Fundamental and Applied Limnology* 177(3): 177–188.
- Fukushima, T., B. Matsushita, W. Yang & L. M. Jaelani, 2018. Semi-analytical prediction of Secchi depth transparency in Lake Kasumigaura using MERIS data. *Limnology* 19: 89–100.
- Fukushima, T., S. Komuro, T. Kitamura, Y. Nagahama & B. Matsushita, 2019. Decadal change in tripton concentration in a shallow lake. *SN Applied Sciences* 1: 1637.
- Fukushima, T., S. Komuro, T. Kitamura, Y. Nagahama & B. Matsushita, 2020. Long-term changes in water mineral concentrations and their influence on sediment water content in a shallow lake. *SN Applied Sciences* 2: 1319.
- Goldman, J. C., J. J. McCarthy & D. G. Peavey, 1979. Growth-rate influence on the chemical composition of phytoplankton in oceanic waters. *Nature* 279(5710): 210–215.
- Goma, R. H., 1995. Dynamics of organic nitrogen in eutrophic Lake Kasumigaura, with special reference to urea and free amino acids. Dissertation to Tokyo University of Fisheries, 142 pp.
- Guildford, S. J. & R. E. Hecky, 2000. Total nitrogen, total phosphorus, and nutrient limitation in lakes and oceans: Is there a common relationship? *Limnology Oceanography* 45(6): 1213–1223.
- Guo, J. B., C. J. Zhang, G. C. Zheng, J. Xue & L. H. Zhang, 2018. The establishment of season-specific eutrophication assessment standards for a water-supply reservoir located in Northeast China based on chlorophyll-a levels. *Ecological Indicators* 85: 11–20.
- Hakanson, L., A. C. Bryhn & J. K. Hytteborn, 2007. On the issue of limiting nutrient and predictions of cyanobacteria in aquatic systems. *The Science of the Total Environment* 379(1): 89–108.
- Havens, K. E., T. Fukushima, P. Xie, T. Iwakuma, R. T. James, N. Takamura, T. Hanazato & T. Yamamoto, 2001. Nutrient dynamics and the eutrophication of shallow lakes Kasumigaura (Japan), Donghu (PR China), and Okeechobee (USA). *Environmental Pollution* 111: 263–272.
- Hennemann, M. C. & M. M. Petrucio, 2016. High chlorophyll a concentration in a low nutrient context: discussions in a subtropical lake dominated by Cyanobacteria. *Journal of Limnology* 75(3): 520–530.
- Ishikawa, T. & M. Tanaka, 1993. Diurnal stratification and its effects on wind-induced currents and water qualities in Lake Kasumigaura, Japan. *Journal of Hydraulic Research* 31(3): 307–322.
- Kaushal, S. S. & W. M. Lewis, 2005. Fate and transport of organic nitrogen in minimally disturbed montane streams of Colorado, USA. *Biogeochemistry* 74(3): 303–321.
- Kolzau, S., C. Wiedner, J. Rucker, J. Kohler, A. Kohler & A. M. Dolman, 2014. Seasonal patterns of nitrogen and phosphorus limitation in four German lakes and the predictability of limitation status from ambient nutrient concentrations. *PLoS ONE* 9(4): e96065.

- Kohzu, A., S. Matsuzaki, S. Komuro, S. Matsumoto, K. Komatsu, N. Takamura, M. Nakagawa, A. Imai & T. Fukushima, 2019. Regime shift analysis for understanding the water quality dynamics in Lake Kasumigaura. Proceedings of 17th World Lake Conference, 365–367. (in Japanese with English abstract).
- Lewis, W. M. & W. A. Wurtsbaugh, 2008. Control of lacustrine phytoplankton by nutrients: erosion of the phosphorus paradigm. *International Review of Hydrobiology* 93(4–5): 446–465.
- Li, Y. P., C. Y. Tang, Z. B. Yu & K. Acharya, 2014. Correlations between algae and water quality: factors driving eutrophication in Lake Taihu, China. *International Journal of Environmental Science and Technology* 11(1): 169–182.
- Maberly, S. C., L. King, M. M. Dent, R. I. Jones & C. E. Gibson, 2002. Nutrient limitation of phytoplankton and periphyton growth in upland lakes. *Freshwater Biology* 47(11): 2136–2152.
- Maberly, S. C., J. A. Pitt, P. S. Davies & L. Carvalho, 2020. Nitrogen and phosphorus limitation and the management of small productive lakes. *Inland Waters* 10(2): 159–172.
- Matsuoka, Y., T. Goda & M. Naito, 1986. An eutrophication model of Lake Kasumigaura. *Ecological Modelling* 31: 201–219.
- Moss, B., E. Jeppesen, M. Sondergaard, T. L. Lauridsen & Z. W. Liu, 2013. Nitrogen, macrophytes, shallow lakes and nutrient limitation: resolution of a current controversy? *Hydrobiologia* 710(1): 3–21.
- Nakamura, K. & M. Aizaki, 2016. Effects of suspended solid on light attenuation in the shallow and turbid Lake Kasumigaura, Japan: long-term variation of the light attenuation mechanism. *Japan Journal of Limnology* 77: 13–23. (in Japanese with English abstract).
- Pisani, O., J. N. Boyer, D. C. Podgorski, C. R. Thomas, T. Coley & R. Jaffe, 2017. Molecular composition and bioavailability of dissolved organic nitrogen in a lake flow-influenced river in south Florida, USA. *Aquatic Sciences* 79(4): 891–908.
- R Core Team, 2020. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.
- Rast, W. & J. A. Thornton, 2005. The Phosphorus loading concept and the OECD eutrophication programme: origin, application and capabilities. In O'Sullivan, P. E. & C. S. Reynolds (eds), *The Lakes Handbook*, Vol. 2. Blackwell Science Ltd., Malden: 354–385.
- Reynolds, C. S. & P. S. Davies, 2001. Sources and bioavailability of phosphorus fractions in freshwaters: a British perspective. *Biological Reviews* 76(1): 27–64.
- Rios, A. F., F. Fraga, F. F. Perez & F. G. Figueiras, 1998. Chemical composition of phytoplankton and particulate organic matter in the Ria de Vigo (NW Spain). *Scientia Marina* 62(3): 257–271.
- Roelke, D. L., P. M. Eldridge & L. A. Cifuentes, 1999. A model of phytoplankton competition for limiting and nonlimiting nutrients: implications for development of estuarine and nearshore management schemes. *Estuaries* 22(1): 92–104.
- Rowland, F. E., R. L. North, P. McEachern, D. V. Obrecht, T. B. Gurung, S. B. Jones & J. R. Jones, 2019. Phytoplankton nutrient deficiencies vary with season in sub-tropical lakes of Nepal. *Hydrobiologia* 833(1): 157–172.
- Schallenberg, M. & C. W. Burns, 2004. Effects of sediment resuspension on phytoplankton production: teasing apart the influences of light, nutrients and algal entrainment. *Freshwater Biology* 49(2): 143–159.
- Schelske, C. L., F. J. Aldridge & W. F. Kenney, 1999. Assessing nutrient limitation and trophic state in Florida lakes. In Reddy, K. R., G. A. O'Connor & C. L. Schelske (eds), *Phosphorus Biogeochemistry in Subtropical Ecosystems*. Lewis Publishers, Boca Roton, FL: 321–342.
- Schindler, D. W., R. E. Hecky, D. I. Findlay, M. P. Stainton, B. R. Parker, M. J. Paterson & S. E. M. Kasian, 2008. Eutrophication of lakes cannot be controlled by reducing nitrogen input: results of a 37-year whole-ecosystem experiment. Proceedings of the National Academy of Sciences of the USA 105(32): 11254–11258.
- Seki, T., T. Fukushima, A. Imai & K. Matsushige, 2006. Turbidity increase and sediment resuspension in Lake Kasumigaura. Proceedings of Japan Society of Civil Engineers G62: 122–134. (in Japanese with English abstract).
- Somiya, I., H. Sumitomo, H. Tsuno, N. Matsuo & Y. Matsuoka, 1989. Water quality prediction model. In Iwasa, Y. (ed.), *Engineering Limnology*. Sankaido, Tokyo: 299–356. (in Japanese).
- Sondergaard, M., T. L. Lauridsen, L. S. Johansson & E. Jeppesen, 2017. Nitrogen or phosphorus limitation in lakes and its impact on phytoplankton biomass and submerged macrophyte cover. *Hydrobiologia* 795(1): 35–48.
- Steffen, W., K. Richardson, J. Rockstrom, S. E. Cornell, I. Fetzer, E. M. Bennett, R. Biggs, S. R. Carpenter, W. de Vries, C. A. de Wit, C. Folke, D. Gerten, J. Heinke, G. M. Mace, L. M. Persson, V. Ramanathan, B. Reyers & S. Sorlin, 2015. Planetary boundaries: guiding human development on a changing planet. *Science* 347(6223): 11.
- Sturner, R. W., 2008. On the phosphorus limitation paradigm for lakes. *International Review of Hydrobiology* 93(4–5): 433–445.
- Stumm, W., 2003. Chemical processes regulating the composition of lake waters. In O'Sullivan, P. E. & C. S. Reynolds (eds), *The Lakes Handbook*, Vol. 1. Blackwell Science Ltd., Malden, MA: 79–106.
- Takamura, N. & M. Nakagawa, 2012. Phytoplankton species abundance in Lake Kasumigaura (Japan) monitored monthly or biweekly since 1978. *Ecological Research* 27: 837.
- Vandergucht, D. M., J. M. Sereda, J. M. Davies & J. J. Hudson, 2013. A comparison of phosphorus deficiency indicators with steady state phosphate in lakes. *Water Research* 47(5): 1816–1826.
- Vollenweider, R. A. & J. Kerekes, 1980. The loading concept as basis for controlling eutrophication philosophy and preliminary-results of the OECD program on eutrophication. *Progress in Water Technology* 12(2): 5–38.
- WEPA, 2020. Lake Kasumigaura. <http://www.wepa-db.net/policies/cases/kasumigaura/01-1.htm/>. Accessed 1 May 2020.
- Wetzel, R. G., 2001. *Limnology*, 3rd ed. Academic Press, San Diego, CA: 365–366.
- Xiao, J., S. Y. Wang, Z. J. Zhou, Y. Zhang, C. L. Song, Y. Y. Zhou & X. Y. Cao, 2018. An enzymatic mechanism for balancing the stoichiometry of nitrogen and phosphorus in

- a shallow Chinese eutrophic lake. *The Science of the Total Environment* 630: 1071–1077.
- Xu, H., H. W. Paerl, B. Q. Qin, G. W. Zhu & G. Gao, 2010. Nitrogen and phosphorus inputs control phytoplankton growth in eutrophic Lake Taihu, China. *Limnology Oceanography* 55(1): 420–432.
- Yang, W., B. Matsushita, J. Chen & T. Fukushima, 2011. Estimating constituent concentrations in case II waters from MERIS satellite data by semi-analytical model optimizing and look-up tables. *Remote Sensing of Environment* 115: 1247–1259.
- Yuan, L. L. & J. R. Jones, 2020. Rethinking phosphorus-chlorophyll relationships in lakes. *Limnology Oceanography* 65(8): 1847–1857.

**Publisher's Note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.