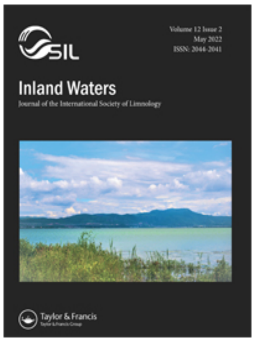

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Phosphorus limitation in low nitrogen lakes in New Zealand

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ABSTRACT
Lakes on volcanic soils of the North Island of New Zealand, compared to north temperate lakes, have low total nitrogen:total phosphorus (TN:TP) ratios, low dissolved inorganic nitrogen concentrations during summer stratification, and have positive chlorophyll *a* responses to N additions more frequently than to P in nutrient enrichment bioassays. This response has resulted, in some cases, to the imposition of N loading caps on some lake catchments, in contrast to nutrient management in north temperate lakes focused more on P management. To explore this possible difference in limiting nutrients, a suite of nutrient status indicators based on measurement of ambient seston stoichiometry and metabolic activity, which have been widely used in north temperate lakes, were applied to 8 lakes on volcanic terrain with previously reported N limitation. These nutrient status measurements were previously calibrated to inform whether in situ phytoplankton are growth-rate limited and, if so, which nutrient is limiting growth rate. In austral summer 2015, all 8 lakes showed indications of P limitation, and P limitation was more extreme, pervasive, and persistent (among samplings) than N limitation. Indications of N limitation were not observed without contemporaneous evidence of P limitation, but P limitation was frequently observed without evidence of N limitation. One lake, Rotorua, was not strongly nutrient limited, and phytoplankton were likely growing at or near optimum growth rates. In this study the commonly used TN:TP ratio was a poor predictor of which nutrient was potentially limiting in situ phytoplankton.

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Introduction

Cultural eutrophication, the addition by human activities of excessive amounts of plant nutrients to receiving waters causing undesirable or noxious algal growth, remains the primary threat globally to beneficial uses of inland and coastal waters (Carpenter et al. 1998, Smith and Schindler 2009, Qin et al. 2020), despite a century of research investigating the effect of nutrients on phytoplankton growth and abundance. At least 18 nutrients are recognized as essential to algal growth, but most are available in most natural waters well in excess of the concentrations required by most phytoplankton for growth (Hecky and Kilham 1988). Only 2 nutrients, nitrogen (N) and phosphorus (P), are generally considered most likely to limit algal abundance in marine and fresh waters. Identifying which of these 2 nutrients is most likely to limit undesirable algal growth in waters of concern is a necessary first step to planning appropriate and efficient control or remediation of eutrophication (Smith and Schindler 2009).

The ratio of total N (TN, sum of all dissolved fixed N and particulate N concentrations) to total P (TP, sum of dissolved and particulate P) is often used to indicate the likelihood of limitation by either nutrient (Abell et al. 2010, Qin et al. 2020). Based on experience in north temperate North American and European lakes, the Organisation of Economic Co-operation and Development (OECD) recommended guidelines for N or P growth limitation as TN:TP <7 weight (16 molar) ratio to indicate N limitation and TN:TP >15 weight (33 molar) ratio to indicate P-limited growth (OECD 1982). Intermediate ratios of TN:TP (i.e., >7 and <15 weight ratio) may be limited by either N or P or both (Forsberg et al. 1978, OECD 1982), or perhaps by some physical parameter (e.g., light). Use of these TN:TP values to indicate N or P limitation have been widely applied (Smith 2006, Qin et al. 2020), including in New Zealand (NZ; Abell et al. 2010).

White (1983) compared nutrient concentrations, including TN, TP, inorganic N, and dissolved reactive P (DRP), in 27 NZ lakes with those in north temperate lakes (OECD 1982) and observed that many NZ lakes had low concentrations of TN relative to TP and strikingly low inorganic N relative to DRP, especially on the North Island of NZ in terrain dominated by volcanic

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soils with high P concentrations in groundwater and springs (Timperley 1983). White (1983, his figure 2) demonstrated that a high proportion (50%) of the surveyed NZ lakes had TN:TP ratios between 7 and 15 (weight) suggesting N and/or P may be potentially limiting by OECD guidelines. This finding contrasted with the north temperate OECD lakes where the vast majority had TN:TP weight ratios in excess of 15, indicative of P limitation. White et al. (1986) conducted a series of N and P nutrient enrichment experiments in 10 North Island NZ lakes in austral summer, and based on the increase in chlorophyll *a* (Chl-*a*) concentration after 4 days of incubation, all responded to N enrichment but not to P. The research by White (1983) and White et al. (1986), although recognizing the potential importance of P, emphasized N as the most likely limiting nutrient in some NZ lakes.

Abell et al. (2010) expanded the TN and TP survey approach of White (1983) to 121 NZ lakes broadly distributed over the North and South islands. TN, TP, and Chl-*a* were reported as annual means based on quarterly or monthly sampling conducted by regional council monitoring programs. The survey concluded that TN was lower for NZ lakes compared to north temperate lakes of similar trophic states up through mesotrophy (based on Chl-*a* concentrations), in general agreement with White (1983). However, Abell et al. (2010) concluded that, based on TN:TP (OECD 1982), P limitation would be most frequent (52% of NZ lakes) while N limitation might be expected in only 17% of lakes, with the remaining lakes (31%) likely to be limited by either N or P or both at the intermediate values of TN:TP. Intermediate values of TN:TP were most frequent in the eutrophic lakes while N limitation was always the least frequently inferred condition, regardless of lake trophic state. Consequently, Abell et al. (2010) concluded that more attention must be paid to P management as well as N to protect NZ waters. The current NZ national policy for freshwater management identifies the need for managing both N and P inputs into lakes (https://environment.govt.nz/acts-and-regulations/national-policy-statements/national-policy-statement-freshwater-management/; accessed 11 Oct 2021), but emphasis on N or P can vary from lake to lake. For example, N loading in Lake Taupō, the largest lake in NZ, is capped, and efforts are in progress to reduce N loading from agricultural sources. While TP concentrations in the lake are monitored to warn against any upward trend in TP, no similar reduction plans are in place for P (Vant 2013; Waikato Regional Plan Water Module; www.waikatoregion.govt.nz/assets/WRC/Council/Policy-and-Plans/Rules-and-regulation/WRP/Chapter-3-Water-Module-Operative-WRP-to-include-NPSFM.pdf; accessed 9 Oct 2021).

For the TN:TP ratio to have mechanistic relevance to algal growth limitation, it must be assumed that all the TN and TP is bioavailable or that the ratio of bioavailable nutrient fluxes of N and P to algae is directly proportional to the TN:TP ratio. If significant proportions of the TN or TP are not bioavailable, then the ratio may be misleading. White (1983) and White et al. (1985) questioned the possible utility of TN:TP in their NZ lakes and suggested that possibly refractory dissolved organic N (DON) in some NZ lakes may accounted for 50–80% of TN. Therefore, the inclusion of refractory N in TN:TP would likely overestimate the bioavailability of N. They reported that a similar, but lesser, effect was also found for the influence of possibly recalcitrant dissolved organic P (DOP) on TP. Consequently, White et al. (1985) suggested correcting TN and TP by subtracting DON and DOP to provide a better estimate of bioavailable N and P, which would reduce the effective TN:TP ratio for many NZ lakes and result in a higher number of NZ lakes considered N limited. However, such a correction has not come into general use and was not applied by Abell et al. (2010), which raises the possibility that their estimate of the number of P-limited lakes, based on TN:TP ratios, may be inflated, and N limitation may indeed be more common in NZ lakes compared to global lakes.

To avoid uncertainty about the bioavailability of TN and TP to algae, a more direct interrogation of the status of nutrient limitation in phytoplankton is preferable. Healey (1973) and Healey and Hendzel (1979, 1980) developed a set of nutrient status indicators based on stoichiometric and metabolic responses in chemostat cultures for a taxonomic range of phytoplankton species. The status of these metrics under conditions of known nutrient limitation and growth rates, induced and controlled in chemostat cultures, enabled Healey and Hendzel (1979) to identify a number of stoichiometric and metabolic assays that could indicate N, P, or general nutrient limitation. Healey and Hendzel (1980) then applied these indicative assays to experimental lakes, where the whole lake responses to defined nutrient additions were known, as well as to natural lakes representing a broad range of trophic and environmental conditions. They concluded that the stoichiometric and metabolic indicators they applied were consistent with the known conditions of N, P, or light limitation that occurred in the studied lakes. The stoichiometric and metabolic indicators developed by Healey and Hendzel have since been applied in a wide range of studies, such as Guildford and Hecky (2000) in global lakes and oceans, Sterner et al. (2004) in

Lake Superior (Canada/USA), Spears and Lesack (2006) in lakes of the MacKenzie Delta (Arctic Canada), North et al. (2007) in Lake Erie (Canada/USA), Gikuma-Njuru et al. (2013) in Lake Victoria (East Africa), Vandergucht et al. (2013) in 109 lakes in Canada, Dubourg et al. (2015) in Lake Diefenbaker (Canada), Higgins et al. (2017) in Lake 227 (Canada), Petty et al. (2020) in 32 midcontinental US reservoirs, and Pothoven and Vanderploeg (2020) in Lake Michigan (Canada/USA). Consequently, the extensive application of the stoichiometric and metabolic phytoplankton nutrient status indicators of Healey and Hendzel (1980) to a diverse set of lakes from a broad range of climatic and trophic conditions provides an extensive database for comparing nutrient status of phytoplankton in other lakes.

In this study we used these broadly applied nutrient status indicators to assess whether N or P or both are most likely to limit phytoplankton growth in a set of lakes in the volcanic highlands of the North Island of NZ, where previously frequent N limitation has been reported based on inference from nutrient concentrations, nutrient ratios, and/or nutrient enrichment bioassays (White et al. 1985, 1986). Our expectation based on this earlier research was that N limitation would be demonstrable during the stratified summer period of minimal dissolved inorganic nutrient concentrations while P limitation would be less frequent and less severe in NZ lakes on the North Island volcanic soils. Independent, confirmatory evidence for the prevalence of N limitation of algal growth by our study would add further support for maintaining or reducing N concentrations in these lakes to control eutrophication in NZ. Conversely, more frequent P limitation in our survey would support the conclusion of Abell et al. (2010) that more attention to P management would be advisable.

Methods

Study area

We chose 8 lakes for our nutrient status survey conducted in austral summer, January–February 2015 (Fig. 1). The lakes are located in close proximity to each other on the North Island of NZ at altitudes between 279 and 418 m in a geologic region called the Taupō Volcanic Zone (TVZ), an area of active volcanism. The lakes offer dramatic physiography because all were created as a consequence of volcanic activity, have catchments with volcanic soils, and contain areas where hydrothermal springs occur in the catchments and lakes. Volcanic soils and springs of the region are rich in P compared to other regions of NZ and globally (Timperley 1983). The lakes are among NZ's most visited tourist attractions; consequently, the lakes are closely monitored to maintain water quality and summer recreational use. The largest lake, iconic Lake Taupō (613 km^2), is in the Waikato Region just north of 3 active volcanoes, Mounts Tongariro, Ruapehu, and Ngauruhoe. The remaining 7 lakes, Õkataina, Rotoiti, Rotoma, Okareka, Rotorua, Tarawera, and Tikitapu, are in the neighboring Bay of Plenty Region. Bathymetries (Table 1) were digitized from NZ Oceanographic Institute Charts (http://www.niwa.co.nz/publications/charts) to calculate volume. Mean depths were calculated by the ratio of volume to lake surface area. All 8 lakes had historic nutrient data and are monitored monthly by regional authorities (Bay of Plenty Regional Council [BOPRC] Environmental Report; BOPRC 2016, Verburg and Albert 2017).

Field observations

The lakes were sampled in austral midsummer 2015 near maximum surface mixed layer (SML) temperatures and during seasonal stratification. Lakes Õkataina, Rotoiti, and Rotoma were visited once; Lakes Okareka, Rotorua, Tarawera, and Tikitapu were visited on 2 dates, 1 week apart; and Lake Taupō was visited 3 times (Table 2). On each sampling date, depth profiles of temperature, in situ fluorescence, and dissolved oxygen (Supplemental Fig. S1) were determined with an RBR XRX-620 profiler (RBR Ltd., Ottawa, Canada); light profiles were determined with a Licor LI-192SA Underwater Quantum sensor (LI-COR Biosciences, Nevada, USA); and Secchi depth transparency was recorded. Rotorua has a monitoring buoy with a thermistor chain (operated by the BOPRC and University of Waikato) that continuously monitors temperatures at several fixed depths over the water column; data from the buoy are accessible to the public (https://envdata.boprc.govt.nz/Data/DataSet/). The buoy also has continuously recording oxygen sensors at the surface and 20 m depth. The buoy and sensors were operative before and during our sampling. The SML was defined using temperature profiles (Supplemental Fig. S1). Water was collected from within the SML (at 10–15 m depth in Lake Taupō and at 5 m depth in the remaining lakes). Lake Taupō water samples were also collected in the metalimnion where oxygen saturation was typically highest (~25 m), and at a depth near the deep Chl-*a* maximum (45–50 m; results from samples below the SML are in Supplemental Tables S1 and S2, but are not discussed here). Carboys (20 L) of water

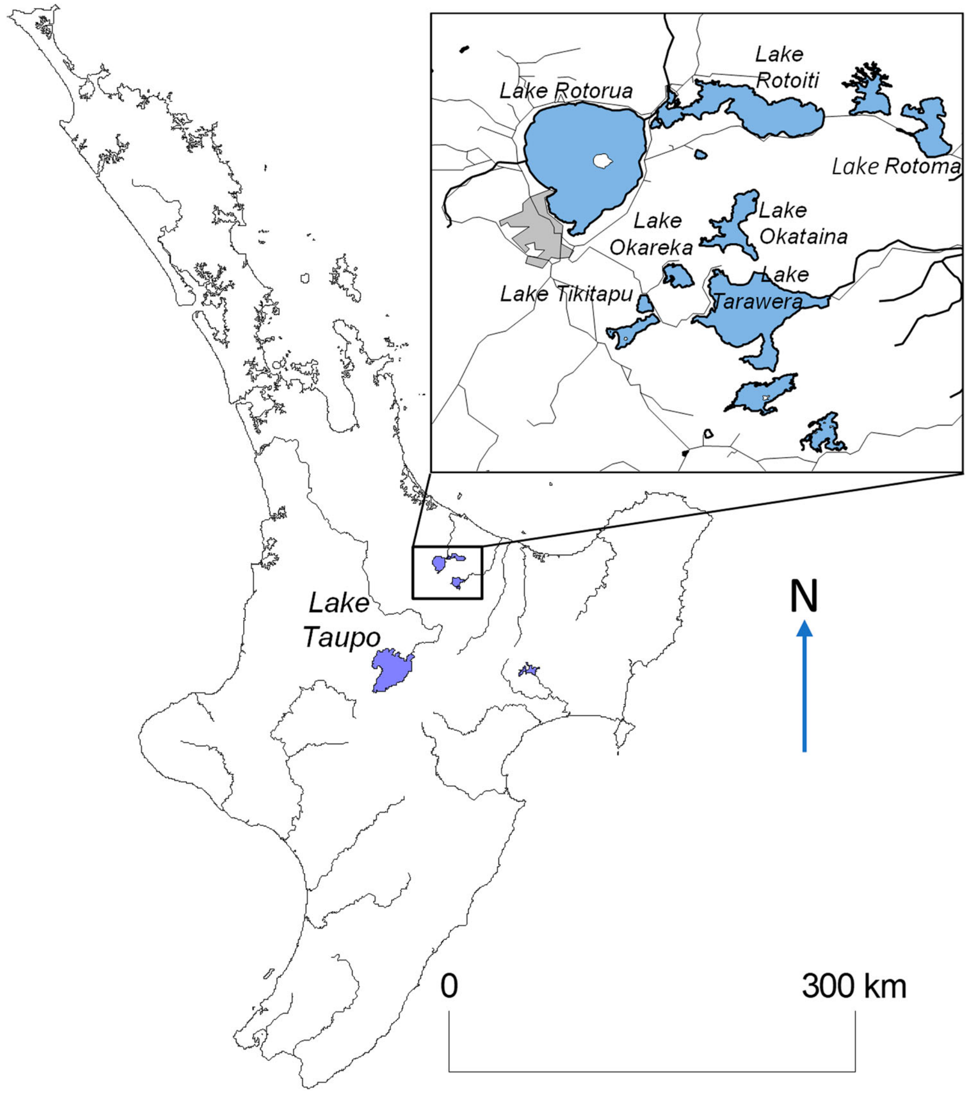

Figure 1. The North Island of New Zealand with locations of Lake Taupō and the Bay of Plenty lakes (inset) sampled in this study.

collected at each sampled depth were kept dark and moved in insulated bins to the laboratory for measurements, which were started the same day. Larger zooplankton was excluded from all water samples by passing water through a 250 μm sieve while pouring into carboys in the field.

Table 1. Physical characteristics of the study lakes and means of water quality parameters collected by the regional councils for the year leading up to this study.

	Area (ha)	Z_{max} (m)	Z_{mean} (m)	Secchi (m)	TP (mg/m^3)	TN (mg/m^3)	NH_4-N (mg/m^3)	NO_3-N (mg/m^3)	TN:TP (wt:wt)	Chl-*a* (mg/m^3)	Trophy
Taupō	61 600	163	92	14.1	5	112	3.3	1.3	21.5	1.3	O
Okataina	1066	79	45	11.0	14	92	1.3	2.1	7.6	1.6	O
Rotoma	1116	83	39	12.6	7	106	2.0	3.9	16.7	1.6	O
Tarawera	4098	88	56	8.1	27	92	1.0	2.6	4.3	1.8	O
Tikitapu	139	28	19	6.3	4	167	1.6	0.9	41.5	1.9	O
Okareka	335	34	19	8.0	10	182	2.5	1.6	19.3	2.7	M
Rotoiti	3354	126	32	7.0	19	164	7.0	3.0	9.5	5.0	M
Rotorua	7862	45	10	3.0	18	283	12.3	12.0	17.1	10.2	E

*Samples were taken using a tube sample. Sample depths were fixed for each lake based on expected depth of the epilimnion and did not change seasonally, which means that at times the sample would have included water from below the epilimnion.

Means are from monthly sampling of the upper water column Jan through Dec 2014, except for Taupō, which was sampled about every 3 weeks. Trophy is trophic status based on Abell et al. (2010); O = oligotrophic, M = mesotrophic, and E = eutrophic. nitrate (NO_3-N) includes nitrite (NO_2-N), which occurred in trace amounts. Z_{max} = maximum depth, Z_{mean} = mean depth, TP = total phosphorus, TN = total nitrogen, NH_4-N = ammonium, Chl-*a* = chlorophyll *a*.

Table 2. Sampling dates (year-month-day), depths, and physical observations during nutrient status surveys in this study.

Lake	Date Collected	Mixed layer depth (m)	Sample depth (m)	Sample depth temperature (°C)	Secchi depth (m)	Light absorption coefficient (per m)	Euphotic zone (m)
Taupō	2015-01-14	11.5	10	20.25	13.5	0.096	48.0
Taupō	2015-01-20	16.4	10	20.73	13.5	0.087	53.0
Taupō	2015-02-11	20.3	15	19.64	13.2	0.100	46.1
Okataina	2015-01-27	10.3	5	22.68	9.0	0.150	30.7
Rotoma	2015-01-27	6.8	5	23.39	11.0	0.133	34.7
Tarawera	2015-01-22	6.7	8	22.30	4.5	0.363	12.7
Tarawera	2015-01-29	9.0	5	22.84	5.2	0.280	16.5
Tikitapu	2015-01-22	7.5	5	22.62	6.5	0.195	23.6
Tikitapu	2015-01-29	7.8	4	22.84	6.1	0.205	22.4
Okareka	2015-01-22	7.2	5	23.26	4.0	0.304	15.1
Okareka	2015-01-29	7.2	5	23.36	3.2	0.283	16.3
Rotoiti	2015-01-27	7.0	5	23.64	8.0	0.231	19.9
Rotorua	2015-01-22	7.9	5	22.75	3.3	0.321	14.3
Rotorua	2015-01-27	5.1	5	24.13	2.5	0.511	9.0

Nutrient analyses

Water samples were analyzed for nitrate (NO_3-N), ammonia (NH_4-N), DRP, Chl-*a*, and particulate C, N, and P. All nutrients were analysed by the Analytical Laboratory of the National Institute of Water and Atmosphere (NIWA) in Hamilton NZ using their standard methods; NO_3-N, NH_4-N, and DRP were measured with a Lachat Flow Injection Analyzer (LaChat Instruments, Milwaukee, WI, USA) following specific QuickChem Methods (https://ca.hach.com/flow-injection-analysis/lachat-quikchem-flow-injection-analysis-system/family-downloads?productCategoryId=22219136200). Detection limits were 0.5, 1, and 0.5 mg/m^3 for NO_3-N, NH_4-N and DRP, respectively. Chl-*a* was determined following filtration through a glass fibre filter (GF/C), extraction in 90% acetone with grinding, and then spectrofluorometric measurement. Samples for particulate C and N were filtered using a precombusted GF/C filter and analyzed using a CHN analyzer. Samples for particulate P were filtered using a GF/C filter, digested with acid and analyzed for DRP. The detection limit was 0.1 mg/m^3 for Chl-*a* and 1 mg/m^3 for particulate C, N, and P.

TN and TP data as well as associated Chl-*a* and NO_3-N concentrations for the year before our surveys were available from the regular monthly monitoring program of the regional councils (BOPRC 2016, Verburg and Albert 2017). These same data sources were also the basis for the analysis of Abell et al. (2010).

Nutrient status measurements

The nutrient status measurements were made using the same metrics, methods, and criteria as used by Guildford and Hecky (2000). The molar ratios of particulate C:N, C:P, N:P, and C:Chl-*a* were calculated from the measurements of particulate C, N, P, and Chl-*a*. The particulate ratios of C:P and N:P can be assessed for P limitation while the C:N ratio can be indicative of N limitation, and the ratio of C:Chl-*a* is considered to be a general (not nutrient specific) indicator of nutrient stress (threshold criteria for the different indicators in Supplemental Table S3).

The metabolic nutrient status indicators were N debt, P debt, and alkaline phosphatase activity (APA). Algae growing at low growth rates because they are deficient in either N or P will take up more of that nutrient per unit Chl-*a* than algae not deficient in that nutrient (Healey and Hendzel 1980). This uptake by N- or P-deficient algae was termed "N debt or P debt" by Healey and Hendzel (1980).

For the N and P debt assays, 200 mL of unfiltered sample was enriched with NH_4Cl or K_2HPO_4 (final concentration of 2 μM N or P). Initial debt subsamples were collected from each flask using a sterile syringe and clean nylon tubing then filtered at 0.45 μm into an acid-washed 50 mL tube and immediately frozen. Incubation flasks were kept in the dark at room temperature for 20 h, at which time the second set of debt subsamples were collected and frozen until analysis of both initial and final subsamples for NH_4-N and DRP concentrations by the NIWA Analytical Laboratory using the methods previously described. N and P debts were calculated as the N or P removed from solution over the incubation period per unit of Chl-*a*.

Phosphatase enzymes associated with P-deficient phytoplankton in lake water act on the added substrate 4-methylumbelliferyl phosphate (4MUP) releasing phosphate and resulting in fluorescence, which was measured in relative fluorescence units with a fluorescence plate reader. The substrate was diluted in 0.2 μm filtered Tris buffer adjusted to pH 8.3 and added to the water sample to achieve a final concentration of 10 μM P. Fluorescence was standardized with 4-methylumbelliferone (4MU), which is naturally fluorescing. Substrate was also added to filtered freshwater media containing no P to monitor any nonbiological production of fluorescence during the assay. The 4MUP substrate was added to all wells except for the standard curve to start the reaction. The reaction temperature was set at 37 °C, and readings were taken every 10 min at excitation/emission wavelengths of 380/454 nm for 60 min. The rate of APA was normalized to Chl-*a*.

Active fluorescence measurements were made with a Walz PhytoPAM (Pulse Amplitude Modulation fluorometer; Heinz Walz GmbH, Germany) after dark adaptation of the sample for 30 min. The quantity F_v/F_m was measured where F_v is the increase in fluorescence over baseline fluorescence F_o and is measured after a strong light pulse, which elucidates maximal fluorescence F_m as the electron transport system is saturated. The ratio is considered to be a measure of photosynthetic efficiency of photosystem II (PSII), which can have a theoretical maximum of 0.65 for dark-adapted samples growing without nutrient stress. Values <0.65 can indicate reduced photosynthetic efficiency and nutrient stress (Parkhill et al. 2001, Juneau and Harrison 2005). Although F_v/F_m was not part of the suite of the other assays validated by Healey and Hendzel (1980) in chemostat cultures and field applications, it has been used as an indicator of nutrient stress by others in field applications (Hiriart-Baer et al. 2008, Guildford et al. 2013, Silsbe et al. 2015). Here, we assumed that F_v/F_m values near 0.65 indicate no or little nutrient limitation and that nutrient stress increases as F_v/F_m declines.

Growth and photosynthetic efficiency of PSII responses to N and P additions

At the end of the last set of N and P debt experiments for each lake (discussed earlier), the incubated flasks with N or P additions were transferred to a controlled environment chamber and exposed to 170 μmol/m^2/s photosynthetically active radiation on a light:dark daily cycle (12:12 h) for 3 days for the Bay of Plenty lakes and 6 days for the Lake Taupō samples. In these growth assays a control consisting of lake water with no nutrient added was run in parallel for each lake. Chl-*a* as relative fluorescence units and photosynthetic efficiency (F_v/F_m) were measured at the beginning and end of the growth assays using the Walz Phyto-Pam fluorometer.

Phytoplankton

Phytoplankton taxonomy and rank abundance were determined on samples from the same sampling depth as the samples (Table 2) for Lake Taupō on 2 visits and on 1 visit for the other 7 lakes (method and results in Supplemental Table S4).

Statistics

Pearson correlation coefficients were determined among all nutrient status parameters after log transformation using Systat 12. Pearson correlations were also determined between Chl-*a* and particulate N and particulate P.

Results

Physical observations

The study lakes were sufficiently deep to stratify in the austral summer (Table 1), although relatively shallow Rotorua (mean depth 10 m) is known to be polymictic and can circulate intermittently during the austral summer months (Burger et al. 2007). However, at the time of our 2 samplings, the lake had been continuously warming and stratified for the previous 6 weeks based on data from the monitoring buoy, and, as a consequence of its eutrophic condition, the hypolimnion had become anoxic at 20 m on January 14, ~1 week before our first sampling. Such stable stratification and hypolimnetic anoxia on Rotorua were also observed in our profiles during our 2 samplings (Supplemental Fig. S1),

Field observations during our 2015 study (Table 2) demonstrated that all the lakes were warm (>20 °C) in the SML and thermally stratified (temperature profiles in Supplemental Fig. S1). The hypolimnetic temperature in Rotorua was >19 °C while all other lakes had hypolimnetic temperatures <14 °C, indicating a weaker stratification in Rotorua than the other lakes. SML depths were as shallow as 6 m in Rotorua to as deep as 20 m in Taupō, and all the lakes were transparent. The euphotic depths (1% of surface light) extended from 9 to >50 m among the lakes and greatly exceeded the SML depth in all the lakes. Rotorua, the shallowest lake, had the lowest ratio (1.8) of euphotic depth to mixed depth. Taupō SML depth descended by nearly 9 m over the 4 weeks of observation (Table 2), although little change in SML temperature was noted in this or the other lakes during our period of study.

Nutrients

In the year prior to our sampling period, the lakes had low mean annual NO_3-N, and NH_4-N concentrations were comparable to NO_3-N (Table 1). Applying the indicative lake trophic status boundaries used by Abell et al. (2010) for mean annual Chl-*a*, 5 of the lakes would be considered oligotrophic, 2 mesotrophic, and 1 eutrophic (Table 1). By the mean TN:TP criteria applied by White (1983) and Abell et al. (2010) (TN:TP < 7 weight [16 molar] indicative of N-limited growth and TN:TP > 15 weight [33 molar] indicative of P-limited growth), 5 of the lakes would be considered P limited, 1 would be considered N limited, and the remaining 2 may be limited by either N or P, or both, or neither (Table 1).

Although annual means are often cited when using TN:TP ratios to infer nutrient limitation in lakes, as in Abell et al. (2010), substantial seasonal variability exists in the ratio among these lakes (Fig. 2). All the lakes had 2- to 3-fold ranges in values over the year prior to our sampling, and all had minimum TN:TP in July–August (austral winter) when the lakes mix to their maximum depths at their coolest temperatures. Although TN:TP values are lowest during seasonal mixing, and 3 of the lakes had TN:TP values inferring N limitation at that time, during restratification TN:TP values increased to higher, fairly stable values by December just prior to our sampling (Fig. 2a), and inferences about P or N limitation for the lakes based on the most recent TN:TP preceding our sampling were nearly the same as that indicated by annual means (Table 1). NO_3-N also exhibited a seasonal pattern (Fig. 2b), with annual maximum concentration values during austral winter deep mixing indicating that, at that time, the demand for N was low

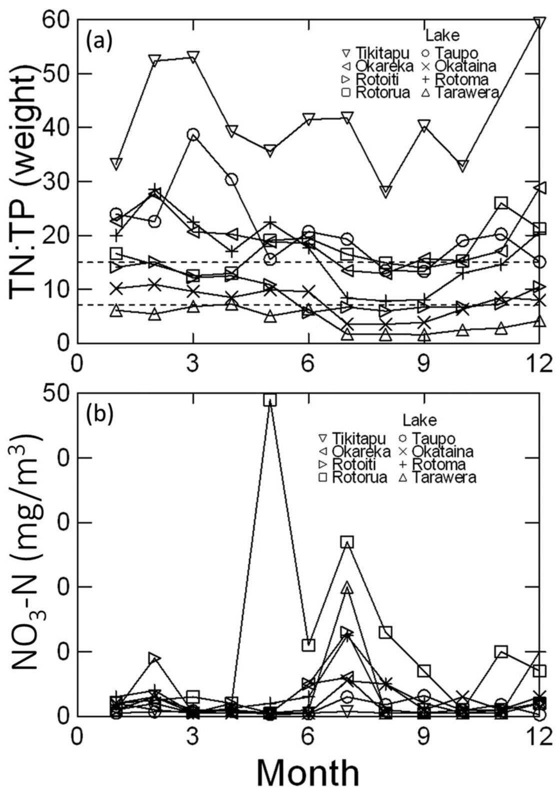

Figure 2. (a) Total nitrogen:total phosphorus (TN:TP) in the 8 study lakes measured monthly during 2014. (b) Nitrate (NO_3-N) concentrations in 2014. Data from the Bay of Plenty Regional Council (2016) and Verburg and Albert (2017).

relative to supply compared to the stratified summer period when NO_3-N was usually not detectable (Table 3). Rotorua exhibited a winter peak in July but also had high NO_3-N concentrations in other months as well as a result of its shallower depth and polymictic behavior (Burger et al. 2007).

Concentrations of NO_3-N in the study lakes (Table 3, Supplemental Table S1) was below the limit of detection (0.5 mg/m^3 N) in all lakes and on all dates sampled during January–February 2015. NH_4-N was also low (<6 mg/m^3 N) but always measurable (Table 3). DRP was at or near detection limits (<0.5 mg/m^3) in all the lakes with the exception of Tarawera, which had DRP concentrations >1 mg/m^3 on both sampling dates (Table 3).

Chlorophyll a and particulate carbon, nitrogen, and phosphorus

Despite the low and often below detection of NO_3-N, NH_4-N, and DRP concentrations in all the lakes at the time of the study, a broad range of Chl-*a* and particulate C, N, and P concentrations was measured (Table 3). Both particulate P and N in the SML of the lakes were highly correlated with Chl-*a* concentrations ($r^2 = 0.83$, $n = 13$, $p < 0.01$, and $r^2 = 0.81$, $n = 14$, $p < 0.01$, respectively). These correlations confirm that phytoplankton biomass, as indicated by Chl-*a*, does increase with particulate N and P, an expected result because both nutrients are required for biomass synthesis. Rotorua had the highest Chl-*a* (Fig. 3a) while Taupō had the lowest, with up to a 20-fold range between these 2 lakes. Similarly, particulate C ranged from highest in Rotorua to lowest in Taupō, but with only a ~7-fold range (Table 3). Rotorua and Taupō were also at the high and low end (respectively) of the ranges of particulate N and P concentrations. Given the low concentrations of dissolved inorganic nutrients, nearly all bioavailable N and P had already been taken into the particulate phase (Table 3), indicating the phytoplankton communities were able to drawdown NO_3-N, NH_4-N, and DRP to near or below detection levels in all the lakes.

Nutrient status

General indicators of nutrient limitation

Both the ratio of C:Chl-*a* and the quantity F_v/F_m can indicate general nutrient limitation. In the surveyed lakes, values of C:Chl-*a* *a* ranged from a low of <8 in Rotorua to >35 in Tikitapu (Fig. 3b, Supplemental Table S2). Elevated values of C:Chl-*a* can indicate limitation by N, P, or some other essential element, and only Rotorua fell below the value indicative of extreme

Table 3. Dissolved inorganic nutrients, chlorophyll *a*, and particulate carbon (C), nitrogen (N), and phosphorus (P) in the surface mixed layer for sampled lakes in this study (Jan–Feb 2015). Date is year-month-day.

Lake	Date Collected	Sample depth (m)	DRP (mg/m^3)	NH_4-N (mg/m^3)	NO_3-N (mg/m^3)	Chl-*a* (mg/m^3)	PC (mg/m^3)	PN (mg/m^3)	PP (mg/m^3)
Taupō	2015-01-14	10	0.6	4	<0.5	0.3	116	17.8	1.4
Taupō	2015-01-20	10	0.7	3	<0.5	0.3	118	12.6	1.5
Taupō	2015-02-11	15	<0.5	2	<0.5	0.7	91.5	12.7	1.1
Okataina	2015-01-27	5	0.5	3	<0.5	1.2	n.d.	n.d.	3.3
Rotoma	2015-01-27	5	<0.5	4	<0.5	0.6	141	22.0	1.9
Tarawera	2015-01-22	8	1.4	1	<0.5	3.5	398	64.8	3.7
Tarawera	2015-01-29	5	1.0	2	<0.5	1.8	378	50.4	2.4
Tikitapu	2015-01-22	5	0.5	2	<0.5	1.1	472	45.6	3.9
Tikitapu	2015-01-29	4	<0.5	1	<0.5	1	434	42.7	3.6
Okareka	2015-01-22	5	<0.5	2	<0.5	1.8	565	58.0	4.0
Okareka	2015-01-29	5	<0.5	4	<0.5	2.0	707	58.8	3.7
Rotoiti	2015-01-27	5	0.5	6	<0.5	1.1	208	28.9	3.9
Rotorua	2015-01-22	5	0.7	4	<0.5	6.9	584	87.6	10.4
Rotorua	2015-01-27	5	<0.5	5	<0.5	8.7	742	101.0	n.d.

DRP = dissolved reactive P, NH_4-N = ammonium, NO_3-N = nitrate, Chl-*a* = chlorophyll *a*, PC = particulate carbon, PN = particulate nitrogen, PP = particulate phosphorus, n.d. = no data.

nutrient limitation (Fig. 3b). Although no validated indicative values inferred nutrient limitation from F_v/F_m, literature values for nutrient-sufficient cultures approach 0.65, with lower values occurring in nutrient-limited batch cultures. Consequently, by this metric we would consider Rotorua to be the least nutrient deficient (average $F_v/F_m = 0.61$; Fig. 3c). On the first 2 visits to Taupō, F_v/F_m values in the SML were not reliably measurable at the lowest ambient Chl-*a* concentrations (0.3 mg/m^3). On the third visit, Chl-*a* was higher (0.7 mg/m^3), and the F_v/F_m was measurable at 0.40, the lowest F_v/F_m observed (Fig. 3c). Considered together, these 2 general nutrient limitation indicators demonstrate that Rotorua was the least nutrient deficient of the lakes. These 2 indicators were also significantly negatively correlated (Supplemental Table S5), as might be expected if they both respond to a common factor.

Indicators of phosphorus limitation

C:P values indicated that all lakes (for which we had complete samples) were moderately or severely P limited on all visits (Fig. 4a, Table 4, Supplemental Table S2). Tarawera, Tikitapu, and Okareka exhibited severe P limitation both times they were sampled. The particulate N:P ratio exhibited a similar relative pattern to the C:P ratio (Fig. 4b), although the 2 lakes with the lowest N:P ratios (Rotoiti and Rotorua) would not be classified as P limited based on the threshold values for N:P (Table 4, Supplemental Table S2). The metabolic indicators of P limitation, APA, and P debt indicated P limitation 100% (13/13) and 77% (10/13) of times sampled, respectively (Table 4, Fig. 4c–d, Supplemental Table S2). Notably, Rotorua was the only lake not to express P debt during our samplings. Good coherence was found between the stoichiometric and metabolic indicators of P limitation, with all well correlated with each other (Table 4, Supplemental Table S5).

Indicators of nitrogen limitation

According to the C:N ratio, Lakes Taupō, Tikitapu, and Okareka were moderately N limited on most or all visits while Tarawera and Rotorua were each moderately N limited on 1 of 2 visits (Fig. 5a). Rotoiti was moderately N limited the one time it was sampled. Unfortunately, the particulate C and N sample for Ōkataina was lost, so no C:N, C:P, or N:P data were recorded for this lake. Based on the available C:N ratios, moderate N limitation was present 69% (9/13) of the times the lakes were sampled (Table 4). Severe N limitation (C:N ratio >14.6 molar, 12.5 mass) was not observed, despite consistently low concentrations of dissolved inorganic nitrogen (DIN) in the study lakes. Limitation by N based on uptake exceeding the threshold for N limitation for the N debt assay was present 29% (4/14) of the times sampled (Fig. 5b, Table 4). N debt values were not significantly correlated with the C:N values or with any other nutrient status indicator; however, C:N was correlated with C:P and the general indicator C:Chl-*a* (Supplemental Table S5).

Healey and Hendzel (1979) observed that under increasing P limitation (e.g., indicated by C:P) in cultured algae, the same cultures also exhibited increases in C:N, so that its use as an indicator of N limitation when C:P is high can be problematic unless supported by other measurements such as N debt. By contrast, C:P was much less sensitive to elevated C:N ratios in N-limited cultures (Healey and Hendzel 1979). In the study lakes (except Ōkataina, where the particulate C and N sample was lost, and Rotorua on the second visit, when the particulate P sample was lost), C:P values

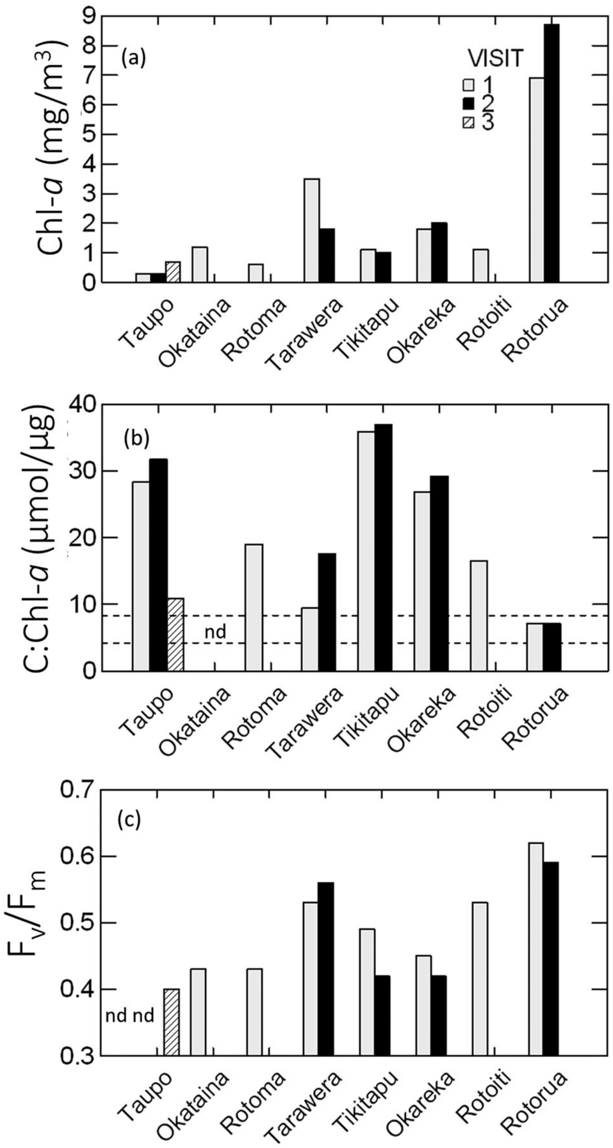

Figure 3. (a) Surface mixed layer chlorophyll *a* concentrations during January–February 2015 (only Taupō visited 3 times). (b) Particulate carbon:chlorophyll *a* (C:Chl-*a*) with dashed lines indicating moderate (lower line) and extreme (upper line) nutrient limitation. (c) Photosynthetic efficiency of photosystem II (F_v/F_m), a general indicator of nutrient limitation. On the first 2 visits to Lake Taupō, F_v/F_m was below detection because of low chlorophyll *a* concentrations. Shading denotes sampling visit number (see Field Observations in Methods section); nd = sample on that visit was lost.

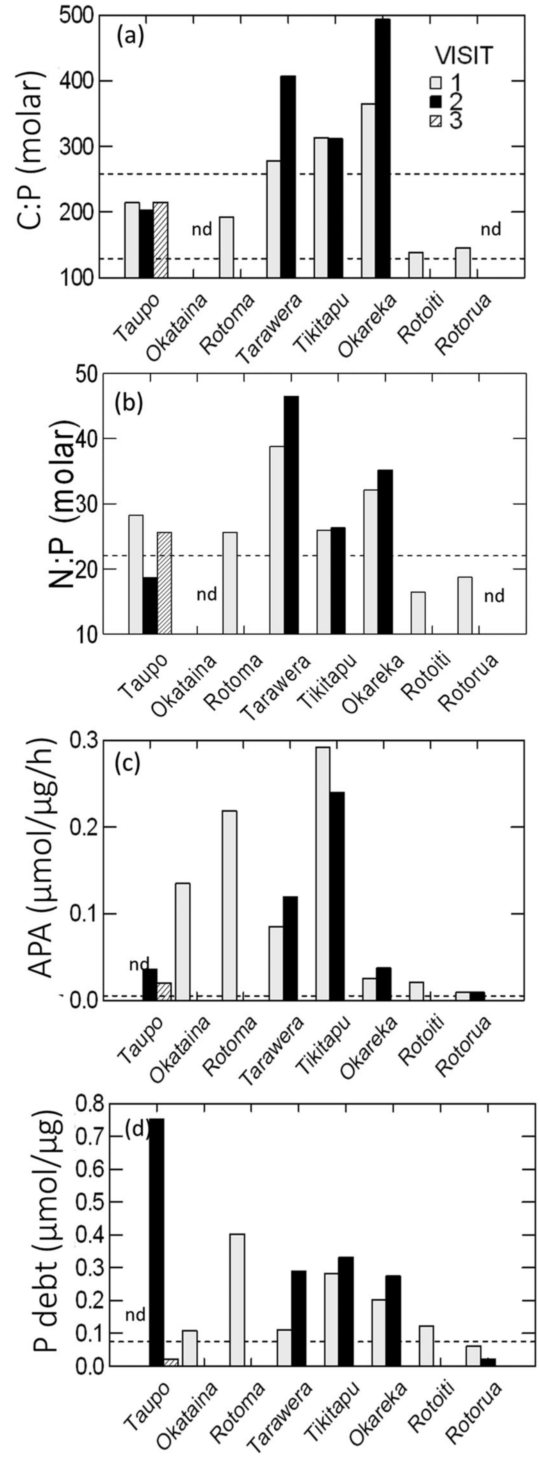

Figure 4. Stoichiometric and metabolic indicators of phosphorus limitation in the surface mixed layer during January–February 2015. (a) Particulate carbon:phosphorus (C:P), dashed lines indicating moderate (lower line) and extreme (upper line) limitation. (b) Particulate nitrogen:phosphorus (N:P), dashed line indicating boundary for P limitation. The C:P sample for Okataina was lost, and the C:P and N:P sample for Rotorua on the second visit was lost. (c) Alkaline phosphatase activity (APA), dashed line indicating severe limitation. (d) Phosphorus debt (P debt), dashed line indicating P limitation. Shading denotes the sampling visit number (see Field Observations in Methods section); nd denotes sample on that visit was lost.

indicated P limitation on all samplings, supported by the metabolic indicator APA in all our samplings and by P debt in all the lakes except Rotorua. C:N and N debt only agreed in 2 of 13 (15%) possible cases (Table 4), and evidence of P limitation was strong in these same cases. No cases were found where N limitation occurred without evidence of P limitation. The low correspondence between C:N and N debt suggests cases indicating moderate limitation by N based on C:N,

Table 4. Nutrient status indicator results for the surface mixed layer of lakes sampled in this study (midsummer 2015). Date is year-month-day.

Lake	Date collected	General limitation		N limitation		P limitation				
		Chl-*a* (mg m^{-3})	C:Chl	N debt	C:N	P debt	C:P	N:P	APA	Overall assessment
Taupo	2015-01-14	0.3	++	0	0	n.d.	+	+	n.d.	P > N
Taupo	2015-01-20	0.3	++	+	+	+	+	0	++	
Taupo	2015-02-11	0.7	++	0	+	0	+	+	++	
Okataina	2015-01-27	1.2	n.d.	+	n.d.	+	n.d.	n.d.	++	P and N
Rotoma	2015-01-27	0.6	++	0	0	+	+	+	++	P > N
Tarawera	2015-01-22	3.5	++	+	0	+	++	+	++	P > N
Tarawera	2015-01-29	1.8	++	0	+	+	++	+	++	
Tikitapu	2015-01-22	1.1	++	+	+	+	++	+	++	P > N
Tikitapu	2015-01-29	1	++	0	+	+	++	+	++	
Okareka	2015-01-22	1.8	++	0	+	+	++	+	++	P > N
Okareka	2015-01-29	2	++	0	+	+	++	+	++	
Rotoiti	2015-01-27	1.1	++	0	+	+	+	0	++	P > N
Rotorua	2015-01-22	6.9	+	0	0	0	+	0	++	P and N
Rotorua	2015-01-27	8.7	+	0	+	0	n.d.	n.d.	++	
% of times deficient			100	29	69	77	100	75	100	

Results in this table are presented as presence (+) or absence (0) of nitrogen (N) limitation for N and phosphorus (P) debt and particulate N:P. The other indicator results are reported as absence (0), moderate (+) or severe (++) limitation. C:Chl (particulate carbon:chlorophyll *a*) can be indicative of general nutrient limitation, while all other indicators are specific for N or P limitation. The percent of times limitation was measured relative to the number of times sampled is given at the bottom of the table. n.d. indicates no data were collected. See methods for detailed explanation of each indicator and Supplemental Table S3 for threshold values for each indicator. See Supplemental Table S2 for numerical data and see Fig. 3–6 for graphs of nutrient status results showing threshold values. Overall assessment column: P > N indicates P debt, and C:P more frequently indicated P limitation than N debt, and C:N indicated N limitation based on summing the number of (+) and (++) for only these indicators. APA is alkaline phosphatase activity.

which was found in 69% of our samples, may be influenced by strong P limitation.

Balance of evidence of nitrogen and phosphorus limitation

If APA and C:P are accepted as surrogates for each other in the few cases where one is absent, then every lake on every sampling date had evidence of at least moderate P limitation by both a metabolic and a stoichiometric measure (Table 4). If comparisons of limitation are made based on particulate C:N and C:P, then moderate N limitation was indicated in 9 of 13 cases, but with no instances of severe N limitation and no instances of C:N indicating N limitation without evidence of P limitation. C:P indications of P limitation were observed in 12 of 12 cases, of which 6 indicated severe limitation. N debt measurements of N limitation occurred in 4 of 14 cases. P debt indicating P limitation was present in 10 of 13 cases. By either stoichiometric or metabolic measurements of nutrient limitation, evidence of P limitation was more prevalent than N limitation in the lakes (Table 4). We did not observe an indication of N limitation without also observing evidence of P limitation (Table 4; the TN:TP ratios from Table 1 were assessed for nutrient limitation in Table 5 according to the criteria of Abell et al. 2010 and the overall assessment in Table 4.).

Focusing only on N and P debt, as comparable metabolic indicators, the results fall into 4 quadrants (Fig. 6) representing N only, P only, N and P dual limitation, and no limitation. The dominance of P limitation is evident by the absence of cases of N-only limitation. Furthermore, the TN:TP ratio (denoted by different symbols in Fig. 6) was a poor predictor of the observed nutrient status limitation. For example, the 4 cases of observed dual limitation spanned the 3 different categories of TN:TP potential limitation, and only 1 case of dual limitation was correctly predicted by the TN:TP ratio (Table 5).

Table 5. Total nitrogen:total phosphorus (TN:TP) by weight from Table 1. Limit (TN:TP) is the nutrient or nutrients predicted to be limiting based on the TN:TP ratio using the criteria of Abell et al. (2010). Limit (nutrient status) is the nutrient or nutrients predicted to be limiting based on the overall assessment from Table 4. P > N indicates that P indicators were dominant compared to N indicators.

	TN:TP (wt:wt)	Limit (TN:TP)	Limit (nutrient status)
Taupō	21.5	P	P > N
Okataina	7.6	P and N	P and N
Rotoma	16.7	P	P > N
Tarawera	4.3	N	P > N
Tikitapu	41.5	P	P > N
Okareka	19.3	P	P > N
Rotoiti	9.5	P and N	P > N
Rotorua	17.1	P	P and N

Growth and photosynthetic efficiency of PSII in N and P enriched samples

Growth assays were conducted on the last round of sampling. Chl-*a* increased over the controls in all P-enriched samples except in Rotorua and Ōkataina (Supplemental Fig. S2). Ōkataina did not respond to either N or P while Rotorua showed modest positive growth in response to N only. In general, samples

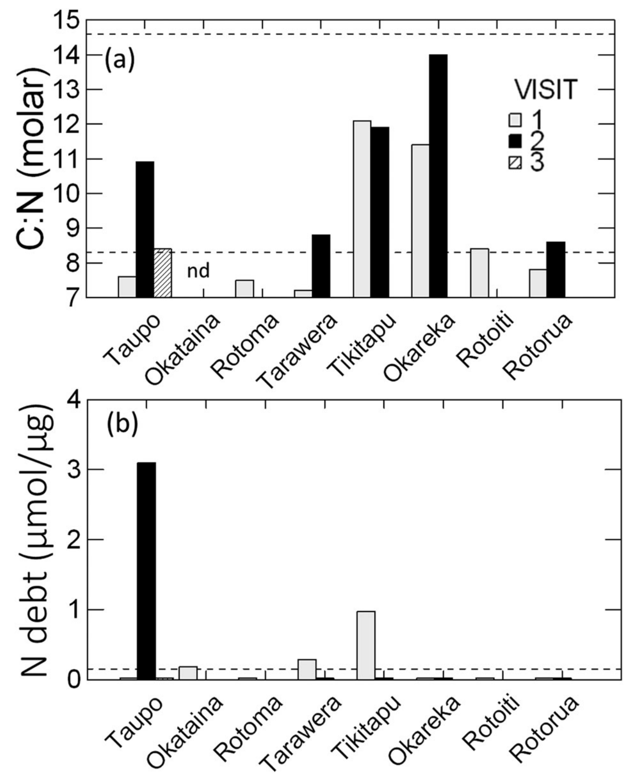

Figure 5. Indicators of nitrogen limitation in the surface mixed layer during January–February 2015. (a) Particulate carbon:nitrogen (C:N) with dashed lines indicating moderate (lower line) and extreme (upper line) limitation. The C:N sample for Okataina was lost. (b) Nitrogen debt (N debt), dashed line indicating boundary for N limitation. Shading denotes the sampling visit number (see Field Observations in Methods section); nd denotes sample on that visit was lost.

amended with N or P had higher F_v/F_m after incubation than unenriched control samples, and the increase was greatest in the P amended samples, with the exception of Rotorua where the control and the N and P amended samples had similar relatively high F_v/F_m values (Supplemental Fig. S3). This positive response of F_v/F_m to nutrient enrichment supports its use as a general indicator of nutrient stress.

Discussion

Expectations and observations

As recommended by White et al. (1986), we sampled for nutrient limitation during the height of austral summer in NZ lakes when dissolved inorganic N and P concentrations are at their annual minima and nutrient limitation is most likely. The studied lakes were transparent with euphotic depths (1% of surface light) substantially deeper than the SML depths. The high transparency and warm temperatures should have eliminated the possibility of light- or temperature-limiting algal growth and nutrient demand, thereby favoring low dissolved nutrient concentrations. These 8 lakes are historically well studied, and N limitation has been reported in all of them. Although White et al. (1985; measurements made in summer 1982) found evidence of P limitation in 2 of these lakes (Rotoma and Okareka), White et al. (1986) concluded that all were N limited based on nutrient enrichment bioassays in summer 1985. Therefore, we expected to find strong evidence of N limitation in at least some of the lakes based on previous studies and nutrient enrichment bioassays in these lakes.

Dissolved nutrient concentrations as indicators of nutrient availability

These NZ lakes had low concentrations of NO_3-N (all samples below detection) during sampling. NH_4-N concentrations were generally measurable but were also low. DRP concentrations were also below or near detection levels in all the lakes, with only Tarawera having DRP in the SML ≥1 mg/m^3. Such low concentrations of inorganic N and P are often invoked to indicate possible nutrient limitation by N or P (e.g., White et al. 1985) but cannot be specifically indicative when both inorganic N and P are so low (Chorus and Spijkerman 2021). Dissolved inorganic nutrient concentrations alone, when so low, are a poor guide to nutrient availability because the rapidity of recycling in the SML is

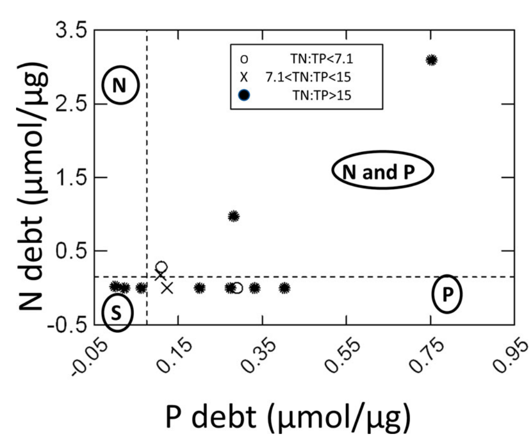

Figure 6. Phosphorus and nitrogen debt (P and N debt) results for all surface mixed layer (SML) samples. Dashed lines indicate the thresholds for P and N limitation (Supplemental Table S3). Symbols denote total nitrogen:total phosphorus (TN:TP) categories for potential nutrient limitation based on Abell et al. (2010). Phytoplankton communities were nutrient sufficient: (S) lower left quadrant, P-only limited (P) lower right quadrant, P and N dual limitation (P and N) upper right quadrant, and N-only (N) limited upper left quadrant. Based on the N and P debt assays, 6 were P-only limited, none were N-only limited, 3 were not nutrient limited, and 4 were limited by P and N.

more important. In austral summer, most of the bioavailable dissolved inorganic N and P in the study lakes was already incorporated into the particulate fractions of N and P, which did vary substantially among the lakes despite similar, and low, dissolved inorganic nutrient concentrations. Therefore, Chl-*a* correlations with particulate nutrients, although similarly significant, cannot be used to determine whether N or P may be limiting phytoplankton growth. Phytoplankton were clearly able to reduce dissolved inorganic nutrient concentrations to below analytical detection while nutrient fluxes remained able to produce and maintain quite variable levels of biomass in these lakes. Consequently, low dissolved nutrient concentrations alone are not useful predictors of growth limitation, with eutrophic Rotorua providing the strongest support for this conclusion because it attained the highest Chl-*a* and particulate nutrient concentration while experiencing low dissolved inorganic N and P concentrations comparable to the more oligotrophic lakes.

Nutrient limitation indicators

Given the low dissolved nutrient concentrations observed in the lakes, nutrient limitation would be expected. The general nutrient limitation indicator, C:Chl-*a*, indicated that phytoplankton in the SML of all the lakes were severely nutrient limited at the time of our sampling, with the exception of Rotorua (and excluding Õkataina where the particulate C sample was lost). Our 2 samplings of Rotorua recorded the lowest C:Chl-*a* and highest F_v/F_m values, both metrics indicating that nutrients were not as strongly limiting as in the other lakes, despite Rotorua's low DIN and DRP similar to the other lakes. F_v/F_m and C:Chl-*a* both indicated a broad range of general nutrient limitation in the lakes. A broad range of Chl-*a* concentrations was also found, with trophic condition extending from oligotrophic (Taupō) to eutrophic (Rotorua), even though DIN and DRP were low in all the lakes. We therefore inferred that fluxes of N and P, in particular by recycling, were more important than static concentrations of inorganic N and P in determining and maintaining phytoplankton biomass and, consequently, that dissolved inorganic nutrient concentrations cannot be used to indicate whether N or P or both are limiting phytoplankton growth in these lakes. A corollary of this inference is that the higher biomass of phytoplankton in Rotorua should have a higher demand for nutrients, and that the flux of nutrients must have met or exceeded the demand to prevent the development of severe nutrient limitation. Vertical mixing of nutrients regenerated at the sediment surface in this large, relatively shallow, weakly stratified lake may provide the requisite higher fluxes, although strong zooplankton grazing pressure might also maintain higher flux rates. In any case, Rotorua stands out for not only having the highest algal biomass among the study lakes but also for being the least nutrient limited.

Nitrogen or phosphorus? The specific nutrient status indicators

All the lakes during all visits had evidence of P limitation by multiple P status indicators, with the exception of Rotorua. The dominance of P limitation in these lakes was clear and repeatable not only among the lakes, but also between sampling visits for those lakes sampled more than once. If we consider a reduced set of P indicators (P debt and C:P) to balance the number of N indicators (N debt and C:N), these indicators exhibited only infrequent (2 lakes, Taupō and Tikitapu on 1 visit each) indications of N limitation by concurrent N debt and C:N. By comparison, 9 cases of both P debt and C:P indicated P limitation concurrently. No case indicated evidence of N limitation without concurrent evidence of P limitation. The relative importance of P was true of the 5 oligotrophic lakes as well the 2 mesotrophic lakes. Oligotrophic Õkataina was only visited once, and only N debt and P debt were measured because the filter for C and N analysis was lost. The debt measurements for Õkataina indicated both P and N would be limiting, indicating dual limitation of the algal community by P and N, a finding supported by the growth assay response in which neither N nor P alone stimulated growth. The overall assessment of the other 6 lakes was that P limitation was dominant (P > N). Only eutrophic Rotorua failed to show strong evidence for P or N limitation, indicating that supply of these 2 nutrients was adequate to meet demand for both nutrients in this lake at the time of our samplings. Consequently, we concluded that algal growth in Rotorua was not strongly limited by either P or N, despite concentrations of dissolved nutrient concentrations below or near our limits of detection. The only indicator of severe P limitation in Rotorua was APA, and it was at the lowest level of all the lakes sampled. Note that our general indicators for nutrient limitation also indicated nutrient sufficiency, which may suggest a physical factor such as light might have been limiting growth in Rotorua.

Because limitation by N had previously been observed in nutrient enrichment growth bioassays in all our study lakes (White et al. 1986), we conducted complementary growth assays (Supplementary Fig. S2, S3) to determine if such assays agreed with the results of the nutrient status measurement. These growth assays also indicated that P limitation was dominant in the lakes, with 6 of 8 lakes responding to added P only (Õkataina and Rotorua were the 2 exceptions). In summary, P limitation, by multiple lines of evidence, was the prevalent form of nutrient limitation, although N limitation was indicated in some of the lakes (e.g., Tikitapu on our first visit), but never without concurrent strong indications of P limitation.

Nutrient status indicators and nutrient enrichment bioassays provide different temporal and environmental perspectives on nutrient limitation. Nutrient status indicators are approximately instantaneous metrics of the compositional and metabolic conditions at the time of sampling. Samples are kept in the dark to prevent photosynthetic growth, and assessments are completed within 24 h. Nutrient enrichment bioassays are time-dependent growth measurements after substantial nutrient enrichment over several days under variable or fixed light conditions. The samples are incubated in bottles under artificial conditions different from the lake (Hecky and Kilham 1988), for example, nutrient enrichment to concentrations orders of magnitude higher than lake concentrations are meant to impose a measurable growth response from a confined sample, but such growth may be different species than were growing under in situ conditions (for instance, Burger et al. 2007, in Lake Rotorua). Considering these differences, the good agreement between the nutrient status indicators and the growth assay responses in our study was noteworthy, but the small sample size reduces confidence in the generality of the result. Some agreement between the 2 methods might be expected but is not necessary given the different measurement conditions. Nutrient enrichment bioassays, by their experimental design, cannot define in situ growth conditions, which are assessed by nutrient status indicators.

Total nitrogen:total phosphorus ratios as indicators of relative nutrient availability

The first question to be answered in a nutrient status assessment is whether nutrients are actually limiting algal growth in the lake; the second question is whether the ambient limitation is by N or P (or some other nutrient, such as a trace metal; Goldman 1964). Both can be answered with the nutrient status indicators, and neither can be answered by nutrient enrichment growth bioassays. The TN:TP ratio, although widely invoked as an indicator of nutrient limitation by N or P (e.g., OECD 1982, Qin et al. 2020), cannot indicate that any nutrient is limiting in situ but can only suggest an answer to the second question once the first has been answered. This conundrum was recognized by its developers (OECD 1982), who clearly indicated, based on the underlying algal assays (Forsberg et al. 1978), that lakes in an intermediate range of TN:TP could be limited by either N or P or both or neither (i.e., limitation would be uncertain based on the ratio alone). OECD (1982) recommended that further biological investigation would be required to establish the limiting nutrient(s) in this range of TN:TP ratios. More recently, Moon et al. (2021) in a large metadata analysis using the US National Lake Assessment database reported 69% of samples had an intermediate TN:TP ratio, complicating any inference about potential nutrient limitations (biomass could be N limited, P limited, N and P colimited, or not limited by nutrients at all). Nutrient status measurements can answer both of the critical questions: Are nutrients limiting in situ phytoplankton growth, and, if so, which nutrient is limiting?

When dissolved inorganic concentrations of one or both N and P are as low as in these 8 NZ lakes, then the likelihood of N or P limitation is high. Thus, we might expect TN:TP to be predictive of limitation by either one or both nutrients. Our surveyed lakes spanned a broad range of TN:TP annual mean weight ratios, from 4.3 to 41.5, with 3 of the lakes falling below 15 weight ratio (33 molar), the boundary above which lakes are considered P limited (OECD 1982), while 5 lakes exceeded it (Taupō, Rotoma, Tikitapu, Okareka, and Rotorua). Only 1 lake, Tarawera, fell into the N-limited range of TN:TP (<7.1 weight) while the 2 other lakes, Õkataina and Rotoiti, were in the intermediate range. However, in our study, Tarawera was assessed as P limited by nutrient status indicators (and the growth assay). Õkataina was shown to be co-limited by N and P by the nutrient status assessment (and the growth assay) while Rotoiti was P limited by nutrient status assessment (and growth assay). The remaining 5 lakes identified as P limited by the TN:TP guidelines of OECD (1982) and the nutrient status assessment concurred, with the exception of Rotorua. Rotorua was expected to be P limited by TN:TP, but no strong limitation was found for N or P by the nutrient status indicators. In summary, the TN:TP ratio correctly predicted the observed limitation by the suite of nutrient status indicators in 5 (Taupō, Õkataina, Rotoma, Tikitapu, and Okareka) of the 8 cases (Table 5). The 3 exceptions occurred across a range of TN:TP ratios from the lowest ratio in Tarawera, where N rather than the observed P limitation was predicted, to Rotorua, which was expected to be P limited by TN:TP but was not assessed to be strongly P limited. If only N and P debts are considered (Fig. 6), the

TN:TP ratio would have predicted only 5 of 13 observations correctly. Although we have only a small number of cases, mismatches between expectations based on TN:TP ratio and observed nutrient limitations were conspicuous. These results demonstrate that whatever utility TN:TP may have in predicting nutrient limitation in large populations of lakes, additional confirmatory nutrient status assessments should be conducted before management action on a specific lake (OECD 1982, Guildford and Hecky 2000, Moon et al. 2021).

Lake Tarawera – the exception and the concern

In summer 2015, Lake Tarawera was exceptional among the lakes with SML DRP >1 mg/m^3 and the second highest Chl-*a* (after Rotorua), although NO_3-N and NH_4-N were as low as the other lakes, and TN:TP was the lowest of the study lakes. Despite having measurable DRP and low TN:TP, indications of P limitation in 2015 were strong, especially in our second sampling. Lake Tarawera was also different from the other lakes because the N-fixing cyanobacterium *Dolichospermum planctonicum* was numerically the dominant algal species. In all the other lakes, neither this species nor other N-fixing species was ranked higher than eighth (rare) in abundance and was usually absent. Nitrogen-fixing taxa may not be limited by N per se, but they can be limited by P, which was the case in Lake Tarawera. However, N-fixing cyanobacteria are not as efficient at competing for DRP and so will be P limited at higher DRP concentrations than other algal taxa (Kenesi et al. 2009). We inferred that low DRP concentrations, as we observed in the other lakes, likely also limit the abundance of N-fixing cyanobacteria in those lakes. The exception of Lake Tarawera, with higher DRP and potential bloom forming, N-fixing cyanobacteria, highlights the importance of controlling P loading to these lakes to keep P concentrations low and limit growth of cyanobacteria as well as other phytoplankton taxa. Controlling N to concentrations even below detection, as in Lake Tarawera, will not be effective in limiting these N-fixing cyanobacteria if sufficient DRP is available. Lake Tarawera illustrates that, even though DIN concentrations are low in our study lakes, they remain prone to the emergence of N-fixing cyanobacteria dominance if DRP concentrations are not maintained at the exceptionally low concentrations that characterize all the lakes in our study, with the exception of Tarawera.

N fixation by cyanobacteria is a response to severe N limitation of the algal community during which non-fixing algal taxa become severely N limited, which restricts their ability to compete for DRP. Consequently, DRP concentration can rise and allow N-fixing cyanobacteria to flourish and dominate the algal assemblage while the non-fixers wane. The seasonal progression of algal community N limitation, evolving to increasing prominence of N-fixing cyanobacteria and eventual P limitation, has been well documented (Hendzel et al. 1994, Higgins et al. 2017, Molot et al. 2021). Our samples from Tarawera observed the terminal phase of this succession as an N-limited algal community became P limited.

Polymictic Rotorua and its recovery

The TN:TP ratio for Rotorua predicted P limitation by OECD guidelines. However, Rotorua was clearly less nutrient deficient than the other lakes in our survey, even though it had the highest algal biomass and an inferred high nutrient demand that kept dissolved inorganic N and P concentrations as low as in the other lakes. We concur with Burger et al. (2007) that polymixis may keep nutrients more available in Rotorua (highest particulate N and P of the lakes surveyed) and in relatively rapid circulation compared to the other stratified lakes. Although it was stratified during our samplings and had been stratified for nearly 6 weeks, the phytoplankton community had not become strongly nutrient limited. In the past, Rotorua was highly eutrophic, but it has undergone a substantial decline in TP, TN, Chl-*a*, and N-fixing cyanobacterial taxa since alum treatments began in 2007 (Smith et al. 2016). Although total nutrient concentrations have fallen in the water column, legacy nutrients in the sediments, when combined with polymixis, likely remain a source of nutrients maintaining a sufficient balanced flux of N and P to prevent development of strong nutrient limitation (Smith et al. 2016). Our results for Rotorua are consistent with Burger et al. (2007), who found weak growth responses to N or P added singly to mixed-layer water samples in nutrient enrichment bioassays in summer 2004. Burger et al. (2007) also developed a dynamic phytoplankton growth limitation model for Rotorua that indicated light could play a major role in limiting algal biomass rather than nutrient availability. These results for Rotorua emphasize that the TN:TP ratio alone as an indicator of nutrient limitation is subject to substantial uncertainties, and a TN:TP ratio cannot indicate that nutrient limitation is actually realized in situ.

Limitations of this study

The 8 lakes in this study are a small subset of the 121 NZ lakes that Abell et al. (2010) surveyed for TN:TP, and our lakes were not selected at random. The lakes were chosen because they had a substantial history of study (BOPRC 2016) and had previously been characterized as exhibiting N limitation (White et al. 1986, Abell et al. 2010). Despite the small sample of NZ lakes, the chosen lakes do span a broad range of TN:TP and trophic status from oligotrophic to eutrophic and therefore can be considered broadly representative of NZ lakes on the volcanic soils of the North Island. We expected that our limited choice of lakes would bias our results toward N-limited lakes because of prior observations, but we observed P limitation to be pervasive and persistent at the time of our study regardless of the lake TN:TP ratios. A broader survey of NZ lakes could be conducted to determine the generality of our nutrient status observations, but our limited set of lakes does indicates that P limitation is likely more frequent in NZ lakes than a historical perspective or the TN:TP ratio would suggest.

Although our study was only conducted in the austral summer, this is the season of minimum nutrient concentrations when nutrient limitation of algal growth would be most likely and also when cyanobacteria are most likely to dominate phytoplankton assemblages (Paerl and Huisman 2008) and to impact user perceptions of water quality. Some NZ lakes, such as Taupō, have austral winter maxima in algal biomass during which N rather than P might limit algal growth (Vincent 1983). We recommend that nutrient status assessments be conducted during all seasons to identify possible seasonal variation in N or P nutrient limitation.

Conclusion

Eight NZ lakes that had been inferred to be N limited in the past were more likely to be P limited than N limited in phytoplankton growth during austral midsummer 2015. Evidence of P limitation was much more pervasive, persistent, and stronger than for N limitation and was virtually independent of the often undetectable dissolved inorganic nutrient concentrations or TN:TP ratios. Although P limitation was pervasive and frequently severe, we did observe evidence of variable N limitation in several of the lakes, suggesting that some of these lakes may be near a balance for the bioavailability of these 2 nutrients. Our results are for the summer period when nutrient limitation should be strongest, and based on our results we concur with Abell et al. (2010) that more effort may be needed to reduce P inputs to protect and remediate NZ lakes and, especially, to prevent cyanobacterial blooms during the summer when cyanobacteria, with their associated aesthetic and harmful effects, are most problematic for human uses (Paerl and Huisman 2008).

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No potential conflict of interest was reported by the author(s).

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Author contributions:

RH, SG, and PV conceived the study; RH, SG, PV, and AA conducted field sampling and laboratory analyses; and RH and SG wrote the manuscript with substantial input from PV.

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