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Assessing nutrient limitation in a subtropical reservoir

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Abstract

There is debate about the relative importance of controlling anthropogenic nitrogen (N) versus phosphorus (P) inputs to limit algal growth in lakes and reservoirs. Our study examined nutrient responses in a subtropical reservoir using short-term algal bioassays on 3 occasions, once during the austral winter and twice during the austral summer. Measurement of photosynthetic yield (Fv/Fm) was used to determine the response to nutrient addition. For the 2 summer sampling occasions, the photosynthetic yields of the N+P treatments were significantly higher than the control. At some sites and on some occasions there was a response to P or N alone, but there was no consistent pattern. The one winter sampling occasion had no response to nutrient addition. Overall, the magnitude of the photosynthetic yield of algal samples correlated with nitrate/nitrite (NO_2^-/NO_3^-) and soluble reactive phosphorus (SRP) concentrations, but not with ammonium (NH_4^+), or dissolved organic N (DON) or P (DOP), despite relatively high concentrations of DON. Therefore we concluded that both N and P co-limited the growth of phytoplankton in the 2 austral summer sampling occasions. This contrasted with high N:P ratios and low P concentrations observed, which suggested that the reservoir was most likely to be P limited. This study highlights the importance of determining algal responses to nutrients and measures nutrient concentrations and ratios to determine whether N or P should be controlled to prevent algal blooms.

Key words: bioassay, nitrogen, nutrient limitation, phosphorus, reservoir

Introduction

Light, nitrogen (N), and phosphorus (P) commonly regulate phytoplankton primary production (Sommer 1989, Litchman et al. 2003). Nutrient limitation can be assessed using a combination of the molar ratio of total N to total P (TN:TP; Redfield 1958, Reynolds 1984) and the absolute concentrations of bioavailable forms of N and P. Under low N and P conditions, aquatic ecosystems with an N:P ratio (molar) less than ~10 are considered to be N deficient, whereas those with N:P ratios (molar) greater than ~20 are considered to be P limited (Grayson et al. 1997). More recently, however, the N:P molar ratio for lakes has been revised to ~21 (Sterner et al. 2008).

The traditional P-limitation paradigm suggests that phytoplankton production in freshwater bodies is more likely to be controlled by phosphate than N availability (Vollenweider 1968, Dillon and Rigler 1974, Schindler 1977), based largely on nutrient addition experiments in temperate North American lakes (Schindler 1974, 1977). However, studies from tropical latitudes have found N more likely to be limiting than P (Vincent et al. 1984, Wurtsbaugh et al. 1985, Dávalos et al. 1989, Lewis 1996, 2002). Contrary to the concept of a single limiting nutrient, the concept of N and P co-limitation has been put forward by various researchers (Lewis and Wurtsbaugh 2008, Sterner 2008). Co-limitation of N and P was found in Lake Pátzcuaro in Mexico (Bernal-Brooks et al. 2003); in 63% of 30 small upland lakes in Cumbria, Wales, Scotland, and Northern Ireland (Maberly et al. 2002); in 2 warm temperate Texan reservoirs (Sterner and Grover 1998, Grover et al. 1999); and in reservoirs in Kansas, USA (Dzialowski et al. 2005). In a study of 8 mountain lakes of central Colorado, 79% of all observed instances of limitation indicated that N was the most frequently limiting nutrient, either alone or in combination with P (Morris and Lewis 1988).

Bioassays are experiments designed to determine the effect of a particular substance on an organism. Nutrient addition bioassays for algal growth in aquatic systems

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are helpful in assessing nutrient limitation (e.g., N, P, or micronutrients) in the water body and to measure the response of phytoplankton communities when the nutrient concentration is increased. Short-term response bioassays, such as the photosynthetic yield response of phytoplankton to nutrient additions, are useful where closed-bottle effects are minimized by having a 24–48 h response time (Ganf and Rea 2007, Burford et al. 2011, Burford et al. forthcoming 2012).

Our study therefore utilized short-term response bioassays to determine nutrient responses of phytoplankton in a subtropical reservoir and compared this with nutrient concentrations and ratios to gain insights into N versus P limitation.

Materials and methods

Study site

Wivenhoe Reservoir (27°24′S, 152°36′E) is situated about 80 km from the city of Brisbane in southeast Queensland, Australia (Fig. 1). The reservoir is 50 km in length, has a storage capacity of 1,165,000 ML, a surface area of 107.5 km^2, a mean depth of 10.8 m (Burford et al. 2007), and a catchment area of 5554 km^2. The climate in the catchment area and the reservoir is subtropical with average monthly rainfall at the dam wall of 75 ± 50 mm in the wet season (Sep–Apr) and 34 ± 26 mm in the dry season (May–Aug; Burford and O'Donohue 2006).

Sampling

Samples were collected in the winter dry season (Aug 2007) and the summer wet season (Nov and Dec 2007) using 0–3 m depth-integrated samplers from 4 sites in Wivenhoe reservoir (Fig. 1; Site 1 is at the dam wall). Samples were poured into 5 L acid washed buckets that were sealed and transported in cool dark conditions to the laboratory. At the same time, subsamples were taken from the bucket for nutrient and chlorophyll *a* concentrations. Unfiltered subsamples were taken for TN and TP analysis. For total dissolved N and P (TDN and TDP), ammonium (NH_4^+), nitrate/nitrite (NO_3^-/NO_2^-), and soluble reactive phosphorus (SRP), the samples were filtered through a 0.45 µm membrane filter. Physical parameters such as water column temperature, dissolved oxygen concentration, pH, conductivity, and turbidity were measured in the surface water at the 4 sites with a water quality monitoring multiprobe meter (YSI 6920 Sonde).

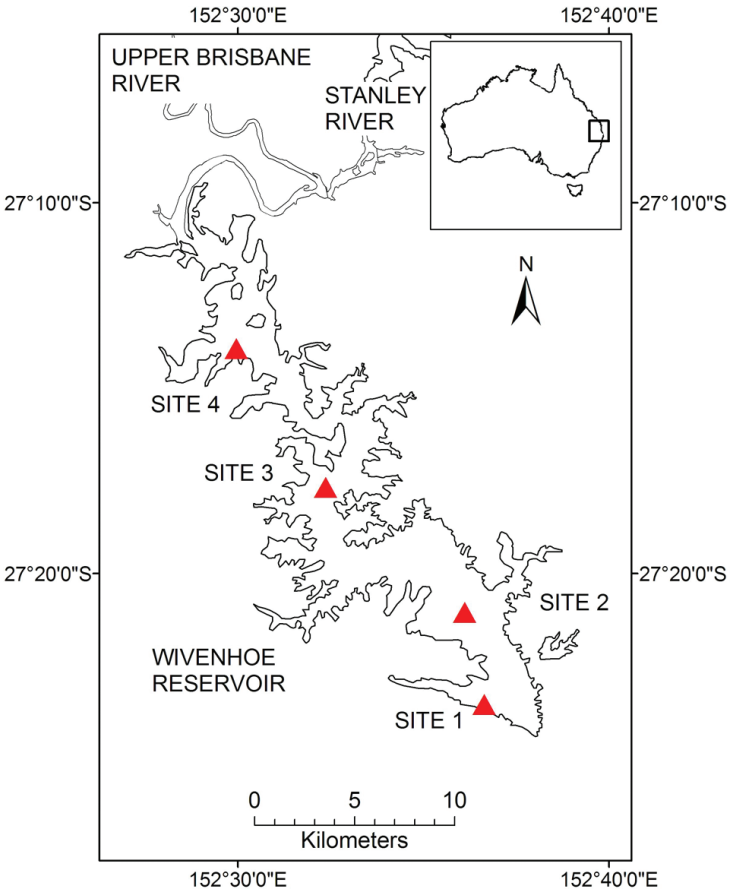

Fig. 1. Wivenhoe Reservoir in southeast Queensland, Australia, showing the 4 sampling sites with Site 1 at the dam wall.

Algal bioassays

The nutrient enrichment bioassays, using water collected in the buckets at each sampling site from the reservoir, were undertaken in closed bottles under controlled light and temperature settings. For each site, 12 clear plastic bottles were filled with 300 mL of water, and 4 treatments were set up in triplicate: control, P only, N only, and N+P addition. The amount of nutrient added to the bioassay bottles was based on concentrations recorded in previous studies of the reservoir (Burford et al. 2007, Muhid 2010). Ammonium chloride was added such that nitrogen was ~60 times background concentrations and potassium dihydrogen phosphate added such that P was ~20 times background concentrations:

$$N = 0.87 \text{ mg } L^{-1} \text{ N final concentration}$$
$$P = 0.062 \text{ mg } L^{-1} \text{ P final concentration}$$

Two subsamples were taken from the buckets for each site and were kept aside in the dark for 20 min to measure

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the background photosynthetic yield using a PHYTO-PAM (Heinz Walz GmbH 2003, Ganf and Rea 2007, Burford et al. 2011, Burford et al. forthcoming 2012), a pulse-amplitude modulation fluorometer that can determine the content of active chlorophyll in waters. Light is pulsed from LED lights resulting in 2 fluorescence readings: F_o as the initial fluorescence and F_m as the maximum fluorescence in response to the light. The variable fluorescence, or yield, is calculated as $F_v = (F_m-F_o)/F_m$, where F_v is the variable fluorescence, F_m is the maximum fluorescence, and F_o is the minimum fluorescence after dark adaptation.

After the nutrients were added, the bottles were placed under fluorescent lights (80 µEin m^{-2} s^{-1}) in a controlled temperature room with a 12 h light:12 h dark cycle. The temperature of the control room was set at the mean temperature measured at the surface of the water column in the reservoir at the time of sampling: 18 ± 2, 24 ± 2, and 27 ± 2 °C for August, November, and December, respectively

The bottles were incubated for 48 h and inverted a number of times to mix the algal community at 24 and 48 h. Upon retrieval after 48 h, the bottles were kept in the dark for at least 20 min before 2 subsamples were taken for photosynthetic yield (activity) measurements using the PHYTO-PAM as outlined above.

Sample analysis

Dissolved inorganic nutrients (SRP, NH_4^+ and NO_3^-/NO_2^-) were analyzed using a Discrete Chemical Analyser (DCA). The detection limits for SRP, NH_4^+, and NO_3^-/NO_2^- were 2, 15, and 3 µg L^{-1}, respectively. TN, TDN, TP, and TDP were digested using a simultaneous persulfate digestion method for N and P (Hosomi and Sudo 1986, Johnes and Heathwaite 1992) and analyzed on a flow injection analyser (LACHAT 8000QC). The concentration of different fractions of N and P were calculated from the results of the above analyses of total and dissolved nutrients. Particulate P (PP) was calculated by subtracting TDP from TP; dissolved organic phosphorus (DOP) was calculated by subtracting phosphate (SRP) from TDP; dissolved organic nitrogen (DON) was calculated by subtracting dissolved inorganic nitrogen (DIN; i.e., $NO_3^-/NO_2^- + NH_4^+$) from TDN; and particulate N (PN) was obtained by subtracting TDN from TN.

Known volumes of the sample were filtered onto 47 mm glass fibre filters (Advantec, GF75) for chlorophyll *a* analysis. Filters were frozen until analysed. Chlorophyll *a* concentrations were determined by extracting glass fibre filters in 100% acetone to maximise extraction efficiency, using a probe sonicator (Branson). Absorbance of these extracts was measured at 750, 665, 664, 647, and 630 nm in 90% acetone; chlorophyll concentrations were calculated according to the method of Jeffrey and Welshmeyer (1997).

Data analysis

A one-way ANOVA with a post-hoc test was performed on the photosynthetic yield data to test for statistical differences between the treatments using the statistical program SPSS (Version 17, SPSS Inc.). A Spearman rank correlation was also performed using SPSS to examine the correlation between the background yields and the nutrient concentrations.

Table 1. Physicochemical parameters measured at the surface at the 4 sites in Wivenhoe Reservoir in August, November, and December 2007; n/a = not available.

Month	Site	Distance from dam wall (km)	Water temperature (°C)	Conductance (µS cm^{-1})	Dissolved oxygen (mg L^{-1})	pH	Turbidity (NTU)	Chl-*a* (µg L^{-1})
August	1	0	16.5	484	9.48	8.3	0.2	n/a
	2	6	16.7	355	8.9	8.1	8.7	n/a
	3	22	16.5	491	9.4	8.3	1.6	n/a
	4	36	16.8	428	8.7	8.2	3.5	n/a
November	1	0	23.8	442	7.9	8.4	0.9	6.9
	2	6	25.6	445	8.9	8.7	0.1	8.7
	3	22	24.7	463	9.1	8.7	2.7	14.0
	4	36	24.9	476	11.8	8.9	3.6	29.6
December	1	0	25.3	457	9.3	8.8	2.3	14.1
	2	6	24.9	461	9.2	8.8	1.6	12.4
	3	22	26.2	481	10.8	9.1	4.3	25.6
	4	36	25.8	486	11.0	8.5	6.1	34.4

Results

Physicochemical parameters and nutrient concentrations in the reservoir

The mean surface water temperature was ~17 °C in August 2007 and ~25 °C in the 2 summer months (Nov and Dec 2007; Table 1). Phosphate concentrations (SRP) and DOP were below or near the detection limit of 2 µg L^{-1} for all 3 sampling occasions. In August (winter) the mean DON (NO_3^-/NO_2^- + NH_4^+) concentration in the reservoir was 180 ± 140 µg L^{-1} compared with 57 ± 78 µg L^{-1} in summer, with Site 4 having the highest concentrations on all sampling occasions (Table 2). DON concentrations were similar at all sites in summer. Site 4 upstream had significantly higher TN, TP, and NO_3^-/NO_2^- concentrations than the downstream Sites 1 and 2 ($p < 0.05$). The TN:TP molar ratios were greater than the molar Redfield ratio of 16 (Redfield 1958) and higher in winter than in summer (Table 2).

Chlorophyll *a* concentrations were higher in December and increased along the longitudinal gradient of the reservoir from the dam wall to upstream (Table 1).

Photosynthetic yields

In August the background photosynthetic yield of water samples at the dam wall was 0.59 ± 0.01 and increased upstream, with Site 4 having the highest background yield (i.e., 0.65 ± 0.01). There was no significant difference observed in the photosynthetic yield of algae with N and P addition after 48 h incubations (Fig. 2; Table 3).

In November, the background photosynthetic yields were similar to August with an increase in yield at upstream sites (Site 1: 0.53 ± 0.02; Site 4: 0.66 ± 0.01). The interaction between site and treatment was significant

Table 2. Mean nutrient concentrations (µg L^{-1}) and molar ratios at the surface of the water column at 4 sites in Wivenhoe Reservoir in August, November, and December 2007; n/a = not available; <2 µg L^{-1} indicates below detection limit.

Site	Month	TN	NO_3/NO_2 -N	NH_4- N	DON	TP	SRP	DOP	TN:TP	DIN:DIP	DON:DOP
Site 1	Aug	540	31	22	n/a	20	< 2	n/a	60	>59	n/a
	Nov	455	16	6	352	21	< 2	3	49	>24	260
	Dec	475	6	3	350	20	2	2	54	10	387
Site 2	Aug	690	44	23	n/a	30	< 2	n/a	51	>74	n/a
	Nov	490	9	4	377	23	< 2	4	48	>14	209
	Dec	480	10	14	355	20	2	2	53	27	393
Site 3	Aug	810	130	150	n/a	21	< 2	n/a	85	>310	n/a
	Nov	555	39	3	353	31	< 2	4	40	>46	195
	Dec	655	8	12	365	34	2	3	43	22	269
Site 4	Aug	920	89	220	n/a	47	3	n/a	43	228	n/a
	Nov	795	144	6	390	36	3	3	49	111	288
	Dec	825	160	13	312	45	2	3	41	191	230

Table 3. One-way ANOVA comparing yields of the control and nutrient treatments of bioassays sampled after 48 h incubations in August, November, and December 2007 ($p < 0.05$); ns = not significant. Bold text shows where nutrient addition was greater than the control (C).

Site	August	November	December
1	ns (all treatments)	**N, P, N+P > C**	N, P < C **N+P > C**
2	ns (control and treatments) **N, P > N+P**	**P, N+P > C** N = C	P < C **N, N+P > C**
3	**P > C** N = C N+P < C	P = C **N, N+P > C**	P < C **N, N+P > C**
4	ns (all treatments)	P = C **N, N+P > C**	ns (all treatments)

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($p < 0.05$). Addition of either N or P alone, or N+P resulted in a significant increase of the yields at the dam wall. However, at Site 2 a significant effect ($p < 0.05$) was observed with the addition of P only (Fig. 2; Table 3). Further upstream, Sites 3 and 4 had increased yields with N only and N+P additions, with N+P having the highest yields ($p < 0.05$; Fig. 2; Table 3).

In December, again there was a significant interaction between sites and treatments ($p < 0.05$). The background photosynthetic yield at Site 1 was 0.57 ± 0.01 and increased upstream with Site 4 at 0.72 ± 0.01. At Site 1, N+P addition treatments had the highest yields (Fig. 2; Table 3). At Sites 2 and 3, N addition and N+P addition treatments resulted in higher yields, while nutrient addition had no significant effect on the yields at Site 4 ($p < 0.05$). At the downstream sites, N+P addition resulted in a higher yield than all the other treatments ($p < 0.05$). In treatments with added P, yields were frequently lower than the control or other treatments at all sites (Fig. 2).

There was a significant positive correlation between water column NO_3^-/NO_2^-, SRP, TN, and TP concentrations, and the photosynthetic yield of samples collected from the 3 sampling occasions and 4 sites (Table 4).

Table 4. Spearmans correlation table for water quality parameters vs. background photosynthetic yield for water samples collected on 3 sampling occasions at 4 sites.

	Correlation coefficient (rho)	Sig. (2-tailed)	N
TN	**0.797**	**0.002**	**12**
DON	0.048	0.911	8
NO_2/NO_3	**0.601**	**0.039**	**12**
NH_4	0.540	0.070	12
TP	**0.607**	**0.036**	**12**
DOP	−0.386	0.345	8
SRP	**0.679**	**0.015**	**12**
TN:TP	−0.105	0.745	12

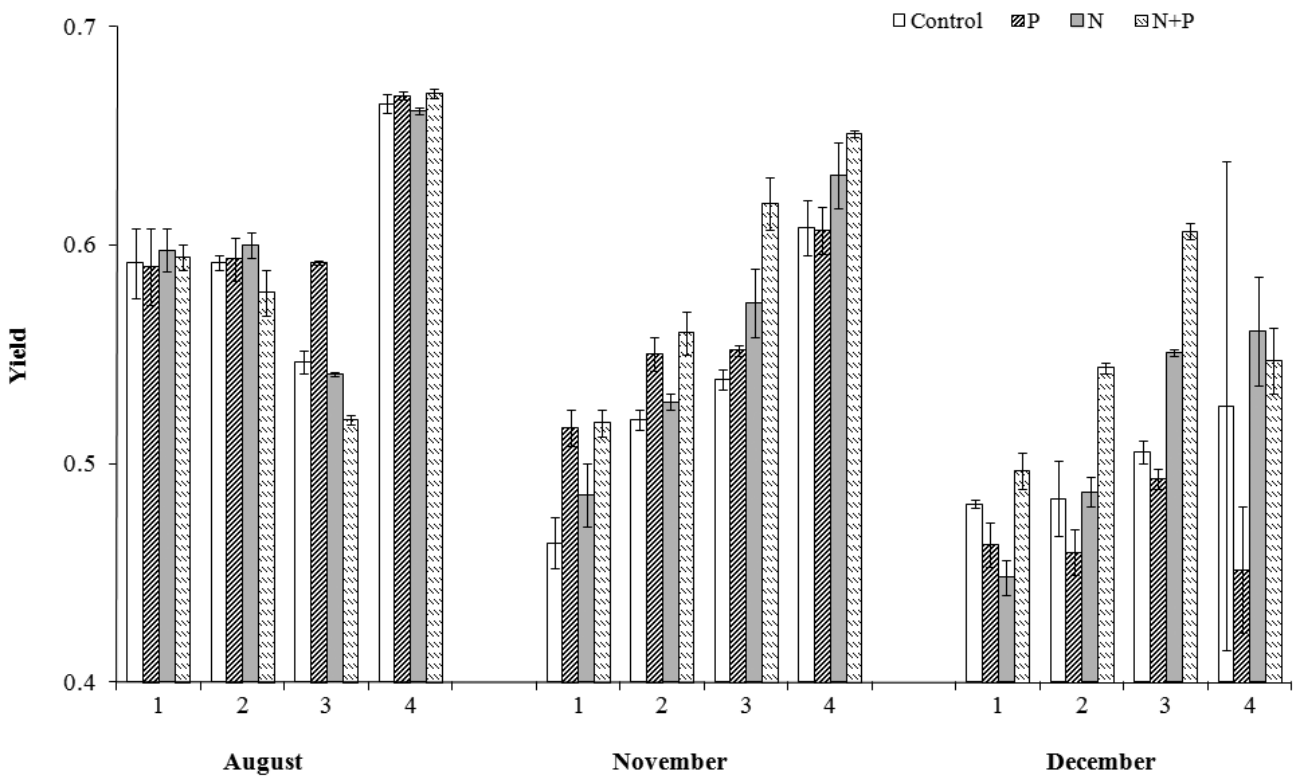

Fig. 2. Mean (± SD) photosynthetic yields of the control and nutrient treatments after 48 h incubations at 4 sites (1, 2, 3, 4) in August, November, and December 2007.

Discussion

This study showed that on the 2 sampling occasions in the austral summer there was evidence of co-limitation of N and P across most sites, despite the N:P ratios often being higher than both Redfield (1958) and Sterner et al. (2008) ratios. This contrasts with a previous study in Wivenhoe, which suggested that the reservoir is more likely to respond to the addition of P rather than N (Burford and O'Donohue 2006) based on relatively high TN:TP ratios and SRP concentrations close to detection limits.

Our results are consistent with a number of other studies indicating nutrient co-limitation. Nutrient enrichment bioassay experiments from 30 small upland lakes in Great Britain showed a high frequency of N limitation or co-limitation at high N:P ratios (Maberly et al. 2002). Similar results were reported from floodplain habitats in Croatia (Peršić et al. 2009) and a river impoundment in the United States (Bukaveckas and Crain 2002). Co-limitation of N+P was also evident in nutrient enrichment bioassay experiments from Lake Tanganyika, Africa (De Wever et al. 2008).

Our study also indicates that a response to N alone, rather than P, is more likely. This was particularly true at the upstream sites that had lower DIN and higher chlorophyll *a* concentrations. Dzialowski et al. (2005) suggested that reservoirs were generally N limited if the water column had TN:TP ratios (molar) <18, co-limited by N and P if TN:TP ratios were between 20 and 46, and P limited if TN:TP ratio >65. This was not the case in Wivenhoe Reservoir, however, where N limitation was seen at sites with TN:TP molar ratios >40.

Ryding and Rast (1989) suggested that during the period of maximum algal biomass there is potential for P limitation if the concentration of biologically available P is <5 µg L^{-1}, N limitation if biologically available N is about 20 µg L^{-1}, and co-limitation if both are less than the given concentrations. Biologically available nutrients include certain forms of DON and DOP; therefore, DOP and DON could also be potential drivers of phytoplankton growth. In SRP-limited conditions, phytoplankton are capable of using phosphatase enzymes to utilise certain forms of DOP (Bentzen and Taylor 1991, Yelloly and Whitton 1996). DON (urea and free amino acids) is also an important N source for phytoplankton (Présing et al. 2001, Berman and Bronk 2003, Burford et al. 2006); however, there was no correlation between DON and DOP concentrations. Background yields in our study indicated that much of the DON and DOP are unlikely to be readily available for phytoplankton growth. Concentrations of biologically available N and P were higher than the levels proposed by Ryding and Rast (1989), and yet co-limitation by both nutrients was measured. Therefore, assessing potential nutrient limitation by examination of nutrient ratios may not be adequate in forming conclusions about nutrient limitation.

Our study showed that spatial and temporal variation in nutrient limitation can occur within the same waterbody. Wivenhoe Reservoir has a longitudinal gradient in terms of nutrient concentrations and phytoplankton biomass declining from the upstream part to the dam wall downstream (Muhid 2010). On the winter sampling occasion when NO_3^-/NO_2^- + NH_4^+ concentrations were higher, there was no response in yield to N, P, or N+P addition, while in summer the addition of N, P, or N+P resulted in higher yields. Other studies have reported highest growth responses of phytoplankton at downstream sites and in late summer, and the interannual variation in nutrient limitation and primary production corresponded to differences in the timing of hydrological inputs and inflow of dissolved nutrients, internal nutrient fluxes, and other biotic factors (Phlips et al. 1993, Havens 1994, López and Dávalos-Lind 1998, Bukaveckas and Crain 2002, Grimm et al. 2003).

Another key reason for the differential impacts of nutrient limitation is that the growth response of phytoplankton is likely to be related to the community composition and competitive ability of species to make use of the nutrient inputs (Hecky and Kilham 1988, Mitrovic et al. 2001, Burger et al. 2007). Rhee (1978) demonstrated that the optimal cellular N:P may be species-specific (i.e., growth of different phytoplankton species may be limited by different nutrients). Differences in species dominance in this reservoir (Muhid 2010) could be a likely reason for the differential responses to nutrient addition observed in this study. In Wivenhoe Reservoir, cyanobacteria are the dominant phytoplankton group by cell concentration throughout the year and by biovolume in the summer months (Burford and O'Donohue 2006, Burford et al. 2007, Muhid 2010). Cyanobacteria are capable of "luxury" uptake of P, and the Nostocales group have heterocysts, specialised cells that can fix atmospheric N. Because N fixation is energetically expensive for the organism, however, biologically available N and NH_4^+, followed by NO_3^-, are the preferred forms for uptake. This was demonstrated in a study by Burford et al. (2006) in an adjacent reservoir, North Pine. The combined abilities of cyanobacteria to store P internally, to withstand low concentrations of external P, and to fix N when dissolved N concentrations are low makes it difficult to determine nutrient limitation without carrying out nutrient addition bioassays. Interestingly, the background photosynthetic yields of the samples were highly correlated with the NO_3^- and SRP concentrations, so these may be the most useful measures of potential nutrient responses.

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In summary this study indicates that co-limitation of N and P occurred throughout a large subtropical reservoir, Wivenhoe Reservoir, in summer months. This contrasted with N:P ratios and nutrient concentrations, including organic nutrients, which did not give an accurate representation of limitation. Nutrient enrichment bioassays therefore provide a potentially more meaningful tool to determine nutrient limitation than nutrient analysis alone. However, to determine if a photosynthetic yield response translates to increased growth, larger-scale and longer-term experiments are needed.

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