Hydrobiologia
https://doi.org/10.1007/s10750-019-3897-8

PRIMARY RESEARCH PAPER

Phytoplankton nutrient deficiencies vary with season in sub-tropical lakes of Nepal

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Received: 22 May 2018 / Revised: 20 January 2019 / Accepted: 23 January 2019

Abstract This is one of few studies to comprehensively examine seasonality and phytoplankton nutrient deficiency in sub-tropical lakes over multiple years, to our knowledge. We tested whether phytoplankton communities in two sub-tropical impoundments in the mid-hills of Nepal were nitrogen (N)-, phosphorus (P)-, or co-deficient in N and P across pre-monsoon, monsoon, and post-monsoon seasons spanning a decade. Nutrient limitation to phytoplankton growth was assessed via in situ stoichiometry of N and P (N:P ratios) and nutrient stimulation experiments (NSEs). The experiments indicated co-deficiency of N and P in 97–100% of NSEs in all seasons. N-deficiency was common ($> 60\%$ of N:P ratios and $\sim 90\%$ of NSEs) during the rainy monsoon, but P-deficiency occurred twice as often as N-deficiency during drier seasons (pre- and post-monsoon). These findings provide perspective for the ongoing debate over N and P as limiting nutrients in freshwater ecosystems by incorporating seasonality and corresponding hydrology. We also re-visit long-standing assumptions regarding the nutrient status of sub-tropical lakes. The warm, stratified season does not fully illustrate lake processes, and more research during dry periods will inform how seasonality affects phytoplankton nutrient deficiencies across aquatic systems.

Keywords Limiting nutrient · Monsoon · Phytoplankton · Nutrient stimulation experiments · Nitrogen · Phosphorus

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Handling editor: David Philip Hamilton

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10750-019-3897-8) contains supplementary material, which is available to authorized users.

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Introduction

Nutrient limitation is a fundamental control on primary production, algal biomass, and community composition (Tilman, 1976). A key aspect of lake science is to understand nutrient regulation of aquatic communities to minimize the negative consequences of eutrophication. There is debate in the literature whether phosphorus (P), or both nitrogen (N) and P ultimately limit freshwater phytoplankton (Lewis & Wurtsbaugh, 2008; Paerl et al., 2016; Higgins et al., 2017). The traditional paradigm suggests primary production in temperate lakes is most strongly controlled by P (Schindler, 1976; Guildford & Hecky, 2000; Schindler et al., 2016), while primary production in tropical lakes is generally considered under N-control (Dussart, 1974; Huszar et al., 2006; Abell et al., 2010; Corman et al., 2015) related to terrestrial processes (Downing et al., 1999). Empirical support for this distinction comes from Huszar et al. (2006) who found the Chlorophyll *a* (Chl *a*)-P relationship weaker among tropical and sub-tropical lakes than the temperate pattern, and from Jones et al. (1989) who reported a strong Chl *a*-N relationship in productive, sub-tropical lakes with low N:P ratios. Numerous studies, however, show N and P co-deficiencies in phytoplankton communities (Elser et al., 2007; North et al., 2007; Sterner, 2008; Lewis & Wurtsbaugh, 2008; Harpole et al., 2011), N-deficiency in temperate lakes (Morris & Lewis, 1988; Abell et al., 2010), and P-deficiency in sub-tropical and tropical lakes (Rai, 2000; Guildford & Hecky, 2000; North et al., 2008). Both N- and P-deficiencies have been measured in tropical Thai reservoirs (Jones et al., 2000), sub-tropical Australian reservoirs (Muhid & Burford, 2012), and the African Great Lakes (Guildford et al., 2003; North et al., 2008).

Sub-tropical and tropical lakes are influenced by seasonal trends (Li et al., 2014), especially in areas affected by monsoons where intense rains result in extreme hydrological events, which contrast sharply with conditions during dry seasons (Lohman et al., 1988; Jones et al., 1989; Jones & Jones, 2002; Corman et al., 2015). Complex interactions of atmospheric deposition, internal nutrient loading, surface runoff, advective mixing, and light limitation make generalizing patterns challenging in monsoon-influenced lakes (Jones et al., 2009). Monsoon rains affect approximately 60% of the world's population (Jones et al., 2009) where both increased drought and intensity of tropical storms are expected with a changing climate (Park & Schubert, 1997; Overpeck & Cole, 2007). Given the extreme variability in global precipitation patterns (Dore, 2005), additional understanding of how seasonality affects nutrient deficiencies to phytoplankton growth in monsoon-influenced lakes is needed.

Seasonal variability in total N (TN) to total P (TP) and particulate nutrient (carbon [C], N, P) ratios in the Nepali lakes Phewa and Begnas Tal have been observed historically in 1985 (Lohman et al., 1988), 1993–1997 (Rai, 2000), and 1997, 1998, and 2000 (Jones & Jones, 2002). In 1985, TN:TP ratios, applied as indicators of potential phytoplankton nutrient deficiencies, indicated N-deficiency [TN:TP < 9 by mass; thresholds from Guildford & Hecky (2000)] in the pre-monsoon months (April–May; Lohman et al., 1988). In the post-monsoon months (September–October), the TN:TP ratios varied between 9 and 23 (Lohman et al., 1988) indicating deficiency in both N and P or another limiting resource. Indicators of N-deficiency were also reported in both the pre- and post-monsoon seasons from 1997 to 2000 (Jones & Jones, 2002). The particulate stoichiometric ratio of year-round (i.e., monthly) samples indicated moderate P- and N-deficiency of the phytoplankton communities (Rai, 2000) using the criteria of Healey & Hendzel (1980). Nutrient ratios were not reported by month or season by Rai (2000), however, preventing detailed evaluation of seasonal nutrient deficiencies to phytoplankton growth.

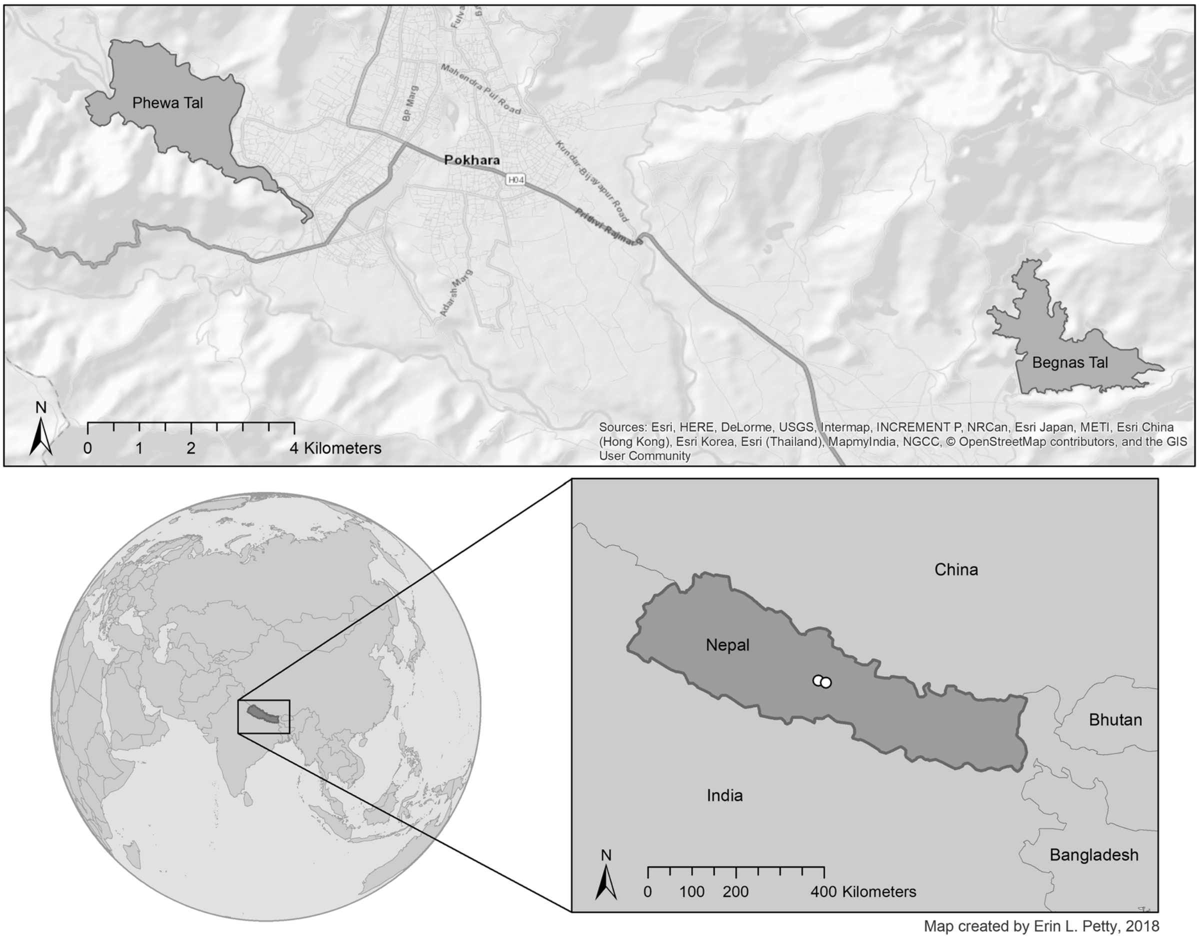
Here we address how seasonality may affect nutrient deficiencies to phytoplankton growth by presenting 86 in situ NSEs in Phewa and Begnas Tal conducted on a year-round, seasonal basis across a decade. We assessed whether the phytoplankton communities in these Nepali lakes, with strong monsoon influence, experienced single nutrient deficiency of either N or P, or co-deficiency of both using year-round water column stoichiometry and in situ nutrient stimulation experiments. We are interested in whether the lakes exhibit different nutrient deficiencies during the monsoon and dry seasons and re-visit long-standing assumptions regarding the nutrient status of sub-tropical lakes.

Materials and methods

Study area

The study lakes, Phewa and Begnas Tal, are warm monomictic sub-tropical lakes located in the mid-hill region of Nepal (Fig. 1). Phewa Tal is a mesotrophic (Table 1; criteria from Jones et al., 2008) natural lake that was formed by gravels and other materials transported from the Annapurna range (Ross & Gilbert, 1999), which has been impounded for hydropower, resulting in a maximum depth of ~ 22 m. It has an area of 4.33 × 10^6 m^2 and a rapid flushing rate, particularly during the monsoon season (Table 2; Sthapit & Leminen, 1992). Begnas Tal, in contrast, has half the volume, a max depth of 11 m (Table 2), and is also mesotrophic (Table 1; criteria from Jones et al., 2008). Begnas, contained by a large dam along the eastern shore, has a flushing rate less than 25% of Phewa (12 vs. 53 times per year, respectively).

We defined seasonality based on precipitation and temperature data from nearby Pokhara airport and further supported by surface water temperatures in both lakes (from 1987 to 2000 in Phewa Tal and from 1989 to 2000 in Begnas Tal), which we use as a proxy for stratification (Fig. 2). Mean annual rainfall from 1984 to 2000 was approximately 4000 mm $year^{-1}$. Rainfall was strongly related to season ($F_{2,\,48} = 277.8$, $P < 0.001$; Fig. 2a). Post-hoc Tukey tests indicated the monsoon season (May–September) had significantly ($P < 0.0001$) higher rainfall than the other seasons. More than 80% of annual precipitation occurs during monsoon season, with the most intense rains in July and August (Fig. 2a). Pre-monsoon (February–April) and post-monsoon (October–January) seasons were not significantly different in rainfall (Tukey test



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Map created by Erin L. Petty, 2018

Fig. 1 Map of Nepal with included lakes Phewa Tal and Begnas Tal

Table 1 Mean values of limnological parameters in Phewa Tal (1987–1998 and 2000) and Begnas Tal (1989–1998 and 2000)

Parameter	Pre-monsoon			Monsoon			Post-monsoon		
	n	Mean	SD	*n*	Mean	SD	*n*	Mean	SD
Phewa Tal									
Secchi (m)	24	4.2	1.1	*86*	*2.7*	*0.7*	31	3.0	0.5
TN (μg l^{-1})	34	248	83	*88*	*209*	*72*	59	239	74
TDN (μg l^{-1})	15	129	47	*52*	*160*	*81*	22	164	71
TP (μg l^{-1})	38	11	3	*93*	*17*	*5*	58	13	3
TDP (μg l^{-1})	15	5	1	*51*	*8*	*2*	21	5	2
TN:TP (mass)	34	25	10	*83*	*14*	*6*	58	19	8
TDN:TDP (mass)	14	29	14	*50*	*23*	*14*	21	38	19
Chl *a* (μg l^{-1})	32	6.2	2.3	*72*	*6.4*	*3.6*	55	13.7	4.5
NVSS (mg l^{-1})	15	0.8	1.3	*49*	*3.0*	*5.2*	33	1.5	1.2
Begnas Tal									
Secchi (m)	11	2.8	0.9	*24*	*3.3*	*0.9*	9	2.2	0.7
TN (μg l^{-1})	13	404	129	*39*	*219*	*75*	17	458	210
TDN (μg l^{-1})	8	280	114	*27*	*145*	*54*	11	400	187
TP (μg l^{-1})	13	14	2	*38*	*11*	*2*	17	15	4
TDP (μg l^{-1})	8	6	2	*27*	*5*	*1*	12	6	1
TN:TP (mass)	13	31	8	*38*	*21*	*7*	17	31	12
TDN:TDP (mass)	8	52	24	*27*	*31*	*16*	11	69	34
Chl *a* (μg l^{-1})	11	7.9	3.0	*34*	*3.8*	*1.3*	17	12.0	6.6
NVSS (mg l^{-1})	8	1.1	0.7	*20*	*0.9*	*0.8*	13	1.0	0.5

All samples were collected from surface waters. For each parameter, we show the number of samples collected (*n*), arithmetic mean, and standard deviation (SD). Monsoon season is italicised for clarity

Table 2 Physical descriptions of Phewa Tal and Begnas Tal

	Phewa	Begnas
Altitude (m)	750	650
Surface area (m^2)	4.33×10^6	3.15×10^6
Watershed area (m^2)	1.10×10^8	1.89×10^7
Volume (km^3)	0.039	0.0023
Maximum depth (m)	23.0	11.0
Mean depth (m)	9.0	6.1
Flushing rate (times year^{-1})	53	12
Siltation rate (m^3 year^{-1})	105,000	18,032

$P = 0.95$), however, air temperature was different (Fig. 2b). Pre- and post-monsoon seasons were defined by periods of warming before (February–April) and cooling after (October–January) the monsoon season (Fig. 2b, c). Both lakes begin to stratify during the pre-monsoon and are typically stratified during the monsoon with periodic disruption by direct rainfall, wind, and inflow during intense storms (occasionally > 100 mm). The post-monsoon season is a period of cooling temperatures and turnover (Rai, 2000). Air temperatures corresponded with surface lake water temperatures; maximum temperatures occurred during the monsoon (Fig. 2b, c). Surface water temperatures in Begnas Tal were consistently ~ 1.5°C higher than the larger lake, Phewa Tal, on a year-round basis (Fig. 2c). We used these seasonal divisions in precipitation and temperature to examine how seasonality may affect nutrient deficiencies to phytoplankton communities in our study lakes.

Experimental set-up

Fifty-four nutrient stimulation experiments (NSEs) were conducted in Phewa Tal during January–December across multiple years (1987, 1990, 1991, 1993–1998, and 2000). The experiments spanned the seasons; 10 were during the pre-monsoon, 30 during the monsoon, and 14 in post-monsoon months. We conducted 32 experiments in Begnas Tal during January–November across multiple years (1989–1991, 1993–1998). In Begnas Tal, we had 6 pre-monsoon NSEs, 21 monsoon season NSEs, and 5

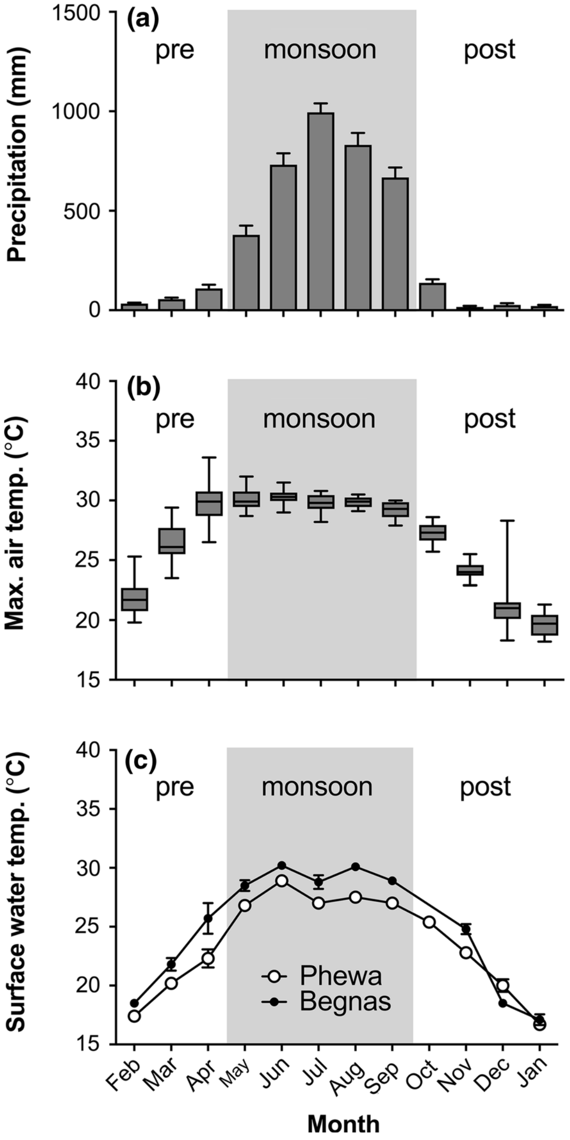


Fig. 2 Climate data from the Pokhara Airport (located in mid-hill region of Nepal) between 1984 and 2000 and lake surface temperatures collected on Phewa between 1987 and 2000 and on Begnas between 1989 and 2000. **a** Bars are monthly precipitation mean ± SE, and **b** boxplots of the mean maximum temperature, and **c** symbols are monthly mean surface water temperature ± SE. Open symbols represent Phewa Tal, and closed symbols are Begnas Tal. Season is denoted by shading and labeling. Note the pre-monsoon season starts in February

post-monsoon NSEs. Our goal was to assess response of Chl *a* (a proxy for phytoplankton) to the addition of N, P, or both, relative to controls.

For each experiment, we used twelve 10 l cubitainers filled with unfiltered surface water. In 1987, we added nutrients at ~ 2× ambient levels, which were 225 μg l^{-1} N as KNO_3 to N-only treatments, and 15 μg l^{-1} P as K_2HPO_4 to P-only treatments, and both to N + P treatments. After 1987, we continued adding nutrients in excess, though at lower concentrations. We added 150 μg l^{-1} N as NH_4NO_3 to N-only treatments, 10 μg l^{-1} of P as K_2HPO_4 to P-only treatments, and both to N + P treatments to test N + P co-deficiency. Control treatments did not receive nutrient additions. Each treatment was replicated 3 times. Three experimental sets, each containing a replicate from each treatment were suspended from anchor lines at half of the Secchi disk depth for 3–7 d in an open area of the lake. At the end of the experiment, we collected the cubitainers and filtered triplicate samples for Chl *a* from each replicate.

We measured Secchi disk depth, N, P, Chl *a*, and non-volatile suspended solids (NVSS) in the surface waters of both lakes at the same time as the NSEs (Table 1; Jones et al., 1989). For N and P analyses, we collected triplicate 10 ml sub-samples of lake water in glass tubes of unfiltered water for total nutrients (TN and TP), and filtrate (1 μm nominal pore size) for the dissolved fraction [total dissolved N (TDN) and total dissolved P (TDP)].

Lab analyses

We filtered the experimental water onto Gelman A/E (1.0 μm nominal pore size) filters in triplicate to assess phaeophytin corrected Chl *a* concentrations as proxies for phytoplankton biomass. Samples were stored in desiccant until they could be transported back to the University of Missouri, Missouri, USA for analysis. We processed Chl *a* filters using the fluorometry methods of Knowlton (1984) and Sartory & Grobbelaar (1986). The N samples were preserved with H_2SO_4 to a pH of 2 or below, and P samples were stored and analyzed in sealed glass tubes. We analyzed N using second derivative analysis of persulfate oxidized samples (Crumpton et al., 1992) and P was analyzed as in Prepas & Rigler (1982). NVSS were measured by filtering a known quantity of water onto a pre-ashed and weighed filter (Whatman 934-AH, 1.5 μm nominal pore size). After drying at 105°C for 1 h, filters were weighed to measure total suspended solids, then ashed at 550°C for 20 min and re-weighed to determine the organic fraction.

Data analysis

We used multiple indicators of nutrient deficiency to phytoplankton growth including in situ total nutrient concentrations and NSEs as recommended by Hecky & Kilham (1988). Ecological stoichiometric theory states that organisms need to obtain elements from the environment in a similar proportion to their own body (Sterner & Elser, 2002). Thus, we considered mass ratios of TN:TP > 23 to be high N:P systems where phytoplankton populations were likely P deficient, and TN:TP < 9 (by mass) were considered N-deficient. Ratios between 9 and 23 indicate neither nutrient nor both could be deficient, or another nutrient or resource- such as light- could be limiting growth (Guildford & Hecky, 2000). For NSEs, we assume that a significant response to the addition of nutrients relative to control indicates deficiency in the nutrient added. We diagnose the nutrient status of phytoplankton communities as the limitation on growth rates (i.e., Blackman limitation; Blackman, 1905). Use of the term nutrient limitation, however, implies ultimate control and cannot be assessed with short-term NSE experiments. Thus, we define nutrient deficiency as the proximate control on instantaneous growth of phytoplankton, and co-deficiency as the simultaneous limitation of phytoplankton growth rates by two or more factors (in this case, N and P). When two or more nutrients are below optimal concentrations, the addition of both will result in an increase in phytoplankton biomass (Arrigo, 2005). Within a phytoplankton community, this type of co-deficiency can be the result of sub-optimal concentrations of one nutrient for one species, and a different deficiency in another algal species (Tilman, 1976).

Most statistical analyses were performed in R (R Core Team 2018). Two-way analysis of variance (ANOVA) on log-transformed Chl *a* concentrations from each experiment allowed us to test how season (pre-monsoon, monsoon, post-monsoon) and nutrient treatment (+P, +N, +N+P) affected Chl *a* relative to controls using the ‘lm’ function in base R. One-way analysis of variance (ANOVA) tests were applied to log-transformed (Chl *a*) values to assess how nutrient treatment may affect algal response relative to controls for each experiment followed by post-hoc Tukey HSD tests to detect significant differences between nutrient treatments using the ‘glht’ function in the ‘multcomp’ package (Hothorn et al., 2008). Initial Chl *a* concentrations were not reported because a paired *t* test indicated initials and controls were not significantly different (Phewa Tal: $t = 1.67$, df = 48, $P = 0.10$; Begnas Tal: $t = -1.15$, df = 28, $P = 0.26$). Residual plots were examined to check the assumptions of normality and heteroscedasticity, and in rare instances, points identified as outliers in Bonferroni outlier tests using ‘outlierTest’ in the ‘car’ package (Fox & Weisberg, 2011) were removed. Pearson correlations calculated in SAS are presented to illustrate cross-seasonal patterns among water quality metrics and water temperature (all probability levels are < 0.01).

Results

Nepali lakes—nutrient inputs

Direct rainfall is the main source of water to both lake basins (Rai, 2000). Water chemistry measured in precipitation samples collected on 4 occasions in 1994 and 1998 contained 1200–7840 µg l^{-1} TN and 35–212 µg l^{-1} TP, resulting in TN:TP ratios between 18 and 42. A single sample of surface flow from a rice paddy in the watershed had 110 µg l^{-1} TN and 12 µg l^{-1} TP (TN:TP of 9). Water samples collected from the main inflowing stream to Phewa Tal, the Harpan Khohla ($n = 19$ samples, not flow weighted), averaged 220 µg l^{-1} TN and 47 µg l^{-1} TP, with an average TN:TP of around 5 (data not shown). Nutrient concentrations in the smaller tributaries to Phewa Tal ($n = 55$ samples, not flow weighted) averaged 260 µg l^{-1} TN and 42 µg l^{-1} TP, with a TN:TP ratio of 9. Inflow streams to Begnas Tal ($n = 18$ samples, not flow weighted) averaged 160 µg l^{-1} TN and 27 µg l^{-1} TP, with a TN:TP ratio of 6.

Water samples collected from a sub-surface spring located ~ 500 m to the east of the boat landing on the north shore of Phewa Tal averaged 1880 µg l^{-1} TN ($n = 41$) and 6 µg l^{-1} TP ($n = 46$); with a TN:TP ratio averaging 483 and a TDN:TN ratio of unity ($n = 24$). The spring flow was oxic, with an average temperature of 23°C. Conductivity of the spring averaged 324 µS ($n = 24$) compared with 46 µS in lake samples ($n = 60$).

Phewa Tal—lake water samples

In Phewa Tal TN averaged 230 μg l^{-1} (Table 1), but varied with season ($F_{2,\ 178} = 4.59$, $P = 0.0114$). Values were significantly lower during the monsoon and higher pre- and post-monsoon (Fig. 3a) such that, across seasons, TN showed a weak negative correlation with water temperature ($n = 224$, $r = -0.29$). Some 10% of TN samples were < 100 or > 360 μg l^{-1}, with these extremes generally measured during the monsoon and post-monsoon. TDN averaged 150 μg l^{-1} (Table 1) and did not vary significantly with season ($F_{2,\ 86} = 1.21$, $P = 0.3023$), although values peaked during the monsoon and post-monsoon (Fig. 4a). TDN averaged about two-thirds of TN, but values at half this average were common in each season. Mean TP was 14 μg l^{-1} (Table 1); 90% of the values were ≤ 20 μg l^{-1} with little seasonal fluctuation except during the monsoon ($F_{2,\ 186} = 13.24$, $P < 0.0001$; Fig. 3a). Across seasons, TP was strongly correlated with NVSS ($n = 162$, $r = 0.89$) but not with TN. About 45% of TP was in dissolved form (Table 1), with monsoon values higher than other seasons ($F_{2,\ 84} = 22.85$, $P < 0.0001$).

TN:TP ratios averaged 20 (Table 1), with about two-thirds of the values between 9 and 23, indicating Phewa was frequently co-deficient in N and P or was

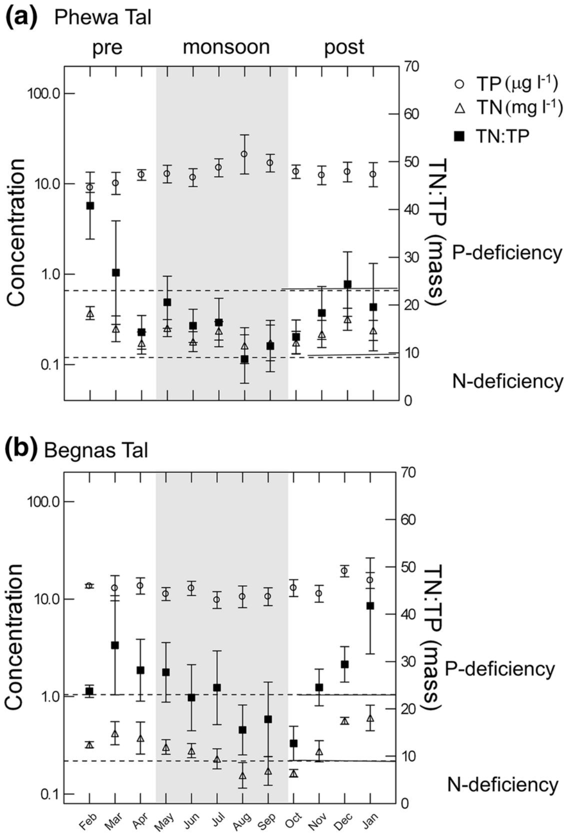


Fig. 3 In-lake nutrient TN:TP (by mass) ratios and nutrient concentrations for **a** Phewa Tal and **b** Begnas Tal. TN:TP > 23 suggests P-deficiency and < 9 suggests N-deficiency (Guildford & Hecky, 2000), indicated on figure with horizontal lines. Ratios between these thresholds indicate either nutrient or both could be deficient. Left hand axes are logged scale. Each point represents the mean and standard deviation for each month from surface water samples. Monsoon season is shaded for clarity

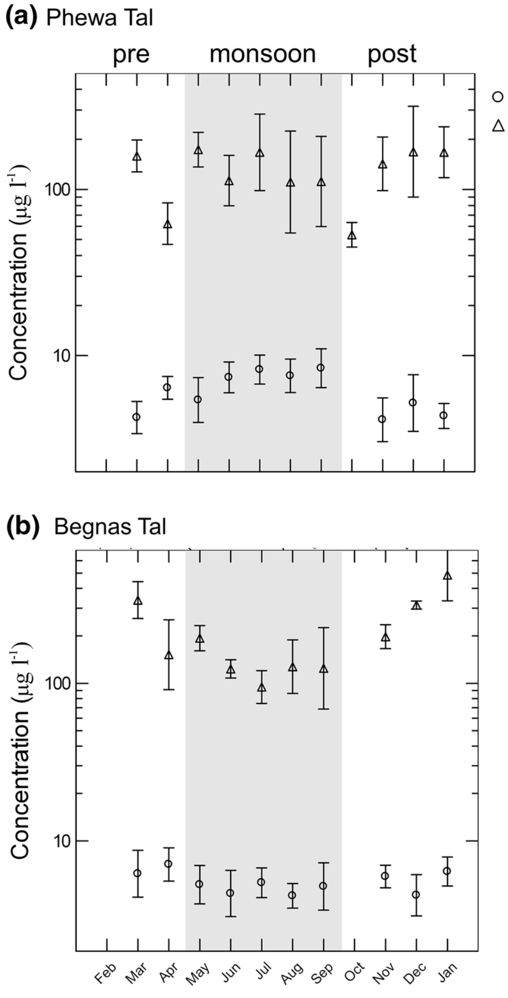


Fig. 4 In-lake dissolved nutrient concentrations for **a** Phewa Tal and **b** Begnas Tal. Left hand axes is logged scale and represents both total dissolved phosphorus (TDP) and total dissolved nitrogen (TDN) concentrations. Each point represents the mean and standard deviation for each month from surface water samples. Monsoon season is shaded for clarity

limited by another nutrient or resource (Fig. 3a). TN:TP ratios significantly varied among seasons ($F_{2,\ 177} = 23.05$, $P < 0.0001$), and averaged 25, 15, and 19 in pre-, monsoon, and post-monsoon samples, respectively (Table 1). Ratios potentially indicated N-deficiency (< 9) in 6% of pre-monsoon, 25% of monsoon, and 3% of post-monsoon samples. P-deficiency (> 23) was potentially indicated in 52%, 10%, and 20% of pre, monsoon, and post-monsoon, respectively (Fig. 3a).

Chl *a* averaged 8.8 μg l^{-1} (Table 1), and was significantly higher post-monsoon, with averages of 6.2, 6.4, and 13.7 μg l^{-1} during the pre-, monsoon, and post–monsoon, respectively ($F_{2,\ 156} = 62.03$, $P < 0.0001$; Table 1). Phewa Tal had a mean Secchi disk depth of 3.3 m, with seasonal variability ($F_{2,\ 138} = 35.21$, $P < 0.0001$). During pre-monsoon, values averaged ~ 4 m, but monsoon and post-monsoon Secchi disk depths were closer to 3 m (Table 1), with individual values from 0.5 to 6 m. Secchi disk transparency was negatively correlated with NVSS ($n = 82$) and TP ($n = 174$) with *r* values of ~ − 0.70.

Phewa Tal—nutrient stimulation experiments

Stimulation of Chl *a* due to nutrient additions varied significantly by season (season x treatment interaction; Table 3), so we conducted additional ANOVAs by season (Fig. 5a, c, e) and for each experiment (Online Appendix Table 1). The pre-monsoon ANOVA indicated a significant effect of nutrient additions on Chl *a* ($F_{3,35} = 10.52$, $P = 0.0004$). Post-hoc tests revealed that N + P addition resulted in a significant ~ 2× increase in Chl *a* relative to controls, but neither N nor P added individually resulted in significant increases (Fig. 5a, Online Appendix Table 1). The monsoon season ANOVA also suggested a significant effect of treatment ($F_{3,111} = 45.19$, $P < 0.0001$). The addition of N increased Chl *a* ~ 2.5×, and N + P increased Chl *a* by more than 3× relative to controls, but P addition alone did not (Fig. 5c). Post-monsoon NSEs had significant treatment effects (ANOVA $F_{3,52} = 4.02$, $P = 0.0120$). N + P addition was the only treatment significantly different from controls, but N and P added independently were not significantly different from the combined addition of N + P (Fig. 5e, Online Appendix Table 1).

During the monsoon, N addition significantly increased Chl *a* in 87% of experiments, 3% of experiments showed a significant increase in Chl *a* with P addition, and 97% of experiments had a significant effect of N + P addition (Fig. 6a, Online Appendix Table 1). Pre- and post-monsoon, however, showed a different pattern. Pre-monsoon, P addition significantly increased Chl *a* in 60% of experiments, compared to N addition, which significantly affected 30% of experiments. N + P addition resulted in a significant increase in Chl *a* in all experiments. Post-monsoon N and P additions significantly increased Chl *a* in 36% and 43% of experiments, respectively, while N + P was significant in 100% of experiments (Fig. 6a, Online Appendix Table 1).

Begnas Tal—lake water samples

In Begnas Tal, TN averaged 360 μg l^{-1}; individual values spanned a tenfold range across the seasons (Table 1, Fig. 3b), with averages of 400 μg l^{-1}, 220 μg l^{-1}, and 460 μg l^{-1} during pre-, monsoon, and post-monsoon samples, respectively. Some 84% of TN measurements during the monsoon were less than the overall average (significantly lower TN; $F_{2,65} = 23.50$, $P < 0.0001$), and across seasons there

Table 3 Two-way ANOVA results for Phewa Tal and Begnas Tal NSEs

Lake	Variable	Sum Sq	df	*F*	*P*
Phewa Tal	Season	18.73	2	45.05	< 0.0001
	Treatment	34.03	3	54.58	< 0.0001
	Season × treatment	5.59	6	4.48	0.0003
	Residual	41.15	198		
Begnas Tal	Season	24.93	2	66.66	< 0.0001
	Treatment	18.16	3	32.38	< 0.0001
	Season × treatment	4.49	6	4.01	0.0011
	Residual	20.94	112		

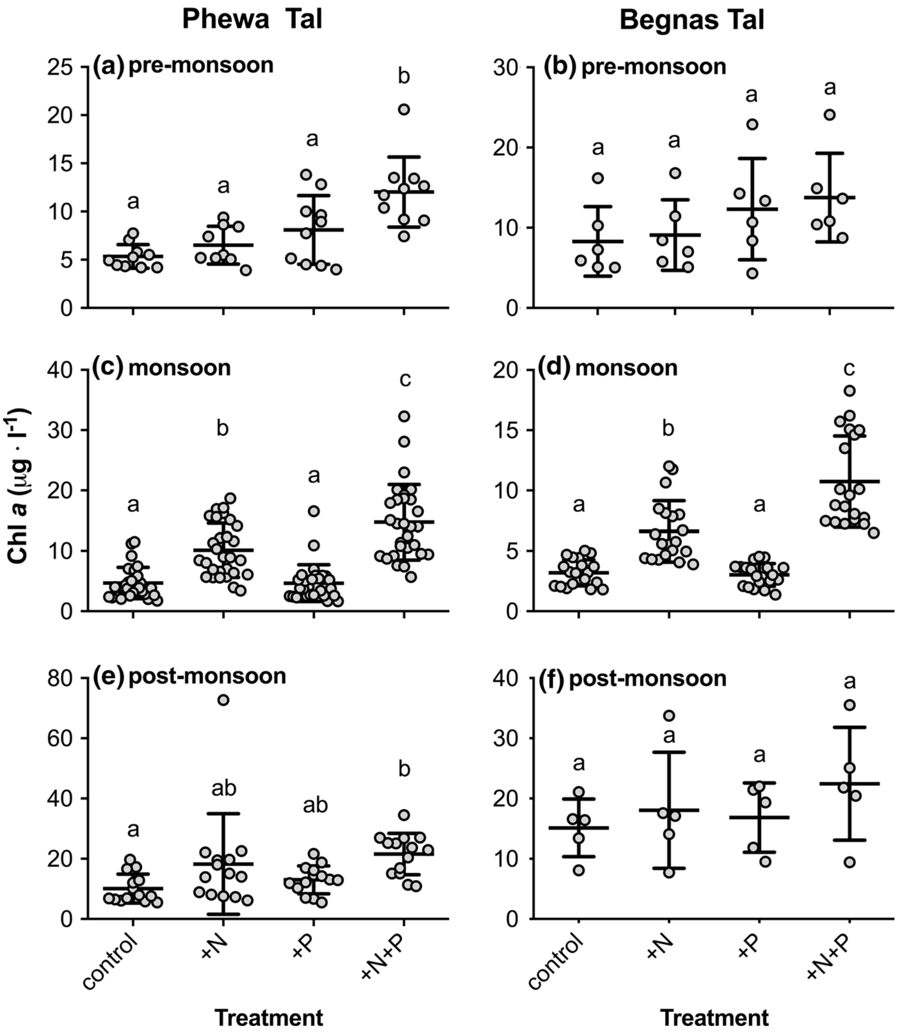


Fig. 5 Summary of NSEs from Phewa Tal (**a**, **c**, **e**) and Begnas Tal (**b**, **d**, **f**) separated by season. Horizontal line represents the mean, and error bars are ± standard deviation. Different letters indicate significant differences in Tukey HSD tests. Note: y-axis changes with season and lake

was a strong negative correlation with water temperature ($n = 98$, $r = -0.76$). TDN averaged 275 μg l^{-1}, with values peaking above average during post-monsoon (Fig. 4b) and significantly lower TDN during the monsoon ($F_{2,43} = 22.06$, $P < 0.0001$; Table 1). TDN averaged about two-thirds of TN; the 2 forms of N were strongly correlated ($n = 116$, $r = 0.89$). Mean TP was 13 μg l^{-1} with values lower in the monsoon relative to other seasons ($F_{2,65} = 10.84$, $P = 0.0009$; Fig. 3b). Across all samples, TP was negatively correlated with water temperature ($n = 98$, $r = -0.60$) and positively correlated with TN ($n = 212$, $r = 0.63$) and NVSS ($n = 82$, $r = 0.43$). About 47% of TP was in the dissolved form (Table 1), with lowest values during the monsoon period, although none of the seasons were significantly different ($F_{2,44} = 2.30$, $P = 0.1124$; Table 1). TN:TP ratios averaged 28, with some 55% of the ratios > 23, suggesting Begnas was frequently P limited (Fig. 3b) and < 5% of the values indicated N-deficiency. During the monsoon, however, 60% of the samples indicated co-deficiency in N and P or control by another resource (between 9 and 23), and TN:TP was significantly lower than in the other two seasons ($F_{2,65} = 11.96$, $P = 0.0004$).

Chl *a* concentrations averaged 7.9 μg l^{-1} (Table 1) with significantly lower values during the monsoon ($F_{2,59} = 42.90$, $P < 0.0001$; seasonal mean 3.8 μg l^{-1}) relative to the pre- and post-monsoon (means 7.9 μg l^{-1} and 12 μg l^{-1}; Table 1). Chl *a* was negatively correlated with water temperature ($n = 93$, $r = -0.64$). Begnas Tal had a mean Secchi disk depth

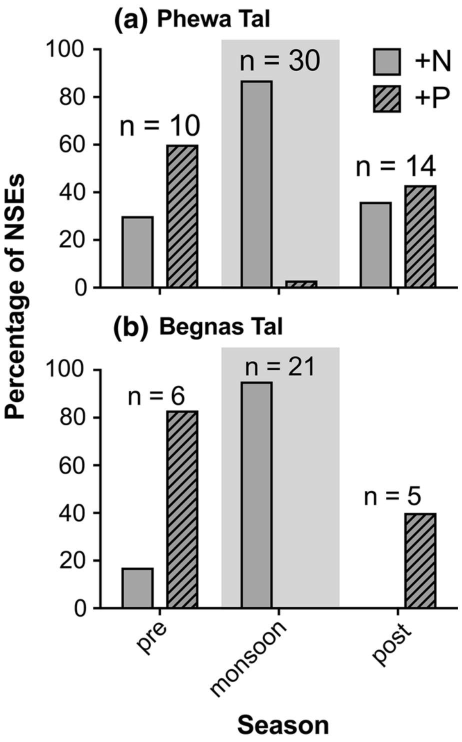


Fig. 6 Percentage of NSEs with significantly higher Chl *a* than controls for **a** Phewa Tal and **b** Begnas Tal. The +N+P treatment significantly increased Chl *a* in 97–100% of experiments, so is excluded to ease the interpretation of +N and +P results. Monsoon season is shaded for clarity, and *n* = number of NSEs included in the calculation

of 2.8 m; transparency was greater than the mean in ~ 60% of monsoon observations and below average in ~ 85% of post-monsoon measurements ($F_{2,41} = 5.69$, $P = 0.0066$; Table 1). There was no relationship between Secchi disk transparency and NVSS but there was a negative correlation with Chl *a* ($n = 61$, $r = -0.60$, both log-transformed).

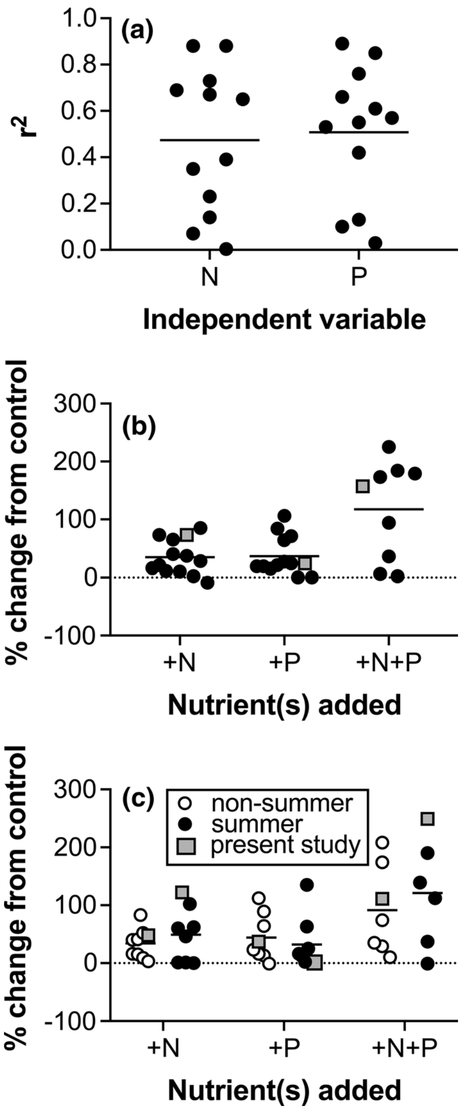
Begnas Tal—nutrient stimulation experiments

Chl *a* response to nutrient additions in Begnas Tal varied with treatment and season (season x treatment interaction; Table 3), so we conducted additional ANOVAs by season (Fig. 5b, d, f) and for each experiment (Online Appendix Table 2). There were no significant effects of treatment during pre-monsoon ($F_{3,20} = 1.74$, $P = 0.192$; Fig. 5b), nor during post-monsoon seasons ($F_{3,19} = 0.89$, $P = 0.463$; Fig. 5f). The monsoon season ANOVA, however, suggested a significant effect of treatment ($F_{3,76} = 61.93$, $P < 0.0001$; Fig. 5d). Post-hoc tests suggested N addition during the monsoon resulted in a significant ~ 2× increase in Chl *a* relative to controls, and N + P significantly increased Chl *a* by ~ 3× relative to controls (Fig. 5d).

N + P addition significantly increased Chl *a* in all experiments among all seasons (Online Appendix Table 2), however, responses to N and P addition varied with season (Fig. 6b, Online Appendix Table 2). During the monsoon, N addition significantly increased Chl *a* in 95% of experiments, while P addition did not result in a significant change in Chl *a* (Fig. 6b). Pre- and post-monsoon NSEs suggested P-deficiency was more common than N-deficiency. Pre-monsoon, P addition significantly increased Chl *a* in 83% of experiments, while only 17% of NSEs had a significant effect of N addition (Fig. 6b, Online Appendix Table 2). Post-monsoon, P significantly increased Chl *a* in 40% of experiments, whereas N addition had no significant effect (Fig. 6b).

Nepali lakes-in context

Regressions between TN, TP, and Chl *a* concentrations (or proxies thereof) and NSEs in tropical and sub-tropical freshwater systems summarized from the literature demonstrate variable responses ($n = 22$; Fig. 7a; Online Appendix Table 3). Relationships between Chl *a* and TN concentrations ranged in r^2 values from 0.004 to 0.88 with a mean of 0.47; similarly, r^2 values for comparisons of Chl *a* and TP concentrations ranged from 0.03 to 0.89 with a mean of 0.51 (Fig. 7a; Online Appendix Table 3). Results from NSEs are comparable, with half of the experiments responding more strongly to P additions, and the other half to N additions (Fig. 7b). Clearly, across this range of systems the variability is stronger than the nutrient response signal. Among most tropical and sub-tropical studies, however, year-round representation is rare and only occurred in 2 of 22 studies, including this one (Fig. 7b, c; Online Appendix Tables 3 and 4). A comparison of NSEs conducted during the summer and non-summer months demonstrated that summer NSEs exhibited a stronger response to the addition of N relative to the addition of P, while the non-summer NSEs closely matched overall averages with a 50/50 split between response to N and response to P additions (Fig. 7c).



◀**Fig. 7** Results of a literature review of some relevant studies of mean annual phytoplankton nutrient deficiencies in tropical and sub-tropical freshwaters. We only included regressions and studies directly testing +N, +P, +N+P treatments. Studies that only compared controls to +N+P or other microelements were excluded. **a** Results from observational studies showing r^2 for regressions between log(Chl *a*) and log(N) (typically log[TN], but also log[DIN] and log[PON] for one study each) or log(TP). **b** Nutrient stimulation experiments (NSEs) are reported with the annual mean increase of nutrient addition relative to control estimated via table values (if available) or digitizing figures in GraphClick 2. The data are reported as % increase or decrease of the control value, such that a value of 15 would indicate that the treatment value was 15% higher than the control. Zero values indicate there was no increase relative to controls. n/a = not applicable because no data were collected on that parameter. Each point represents the mean of a study, and the horizontal line indicates the mean of all the studies. If multiple lakes were included in the study, the data were first averaged among experiments within a lake (if applicable), and then across lakes. **c** Separate studies that collected data in the summer and non-summer seasons. Gray boxes show the results from the current study. We only used values reported in tables or digitized from figures, so these values may have a bias toward significant results. More details on the studies shown here can be found in Online Appendix Tables 3 and 4

Discussion

We found N and P co-deficiency most common (~ 100% of NSEs and ~ 50% of TN:TP ratios) in these warm monomictic lakes in the humid sub-tropics. Our results strongly support N-deficiency during the monsoon, with a significant response in ~ 90% of NSEs to the addition of N, similar to the paradigm for tropical lakes (Dussart, 1974; Downing et al., 1999; Huszar et al., 2006; Abell et al., 2010). During the monsoon, N-deficiency occurred in 26 of the 30 NSEs in Phewa Tal, and not a single NSE indicated P-deficiency in Begnas Tal (zero of 21 NSEs; Fig. 6). This response is consistent with low surface water N concentrations and low TN:TP ratios in both lakes (Figs. 3, 4) and their tributaries, although N in direct rainfall may have been an additional source of this nutrient. Seasonally, however, the pattern switches, likely due to lake processes tied to circulation (Figs. 5, 6). Pre- and post-monsoon, NSEs in both lakes indicated P-deficiency. During the pre-monsoon (February through April), when both lakes transition from holomixis to stratification (Fig. 2c), P-deficiency was twice as common as N-deficiency in Phewa Tal and ~ 5× more common in Begnas Tal (Fig. 6). During the post-monsoon (late September through January) when the lakes became holomictic again (Fig. 2c), P and N-deficiency were relatively equivalent in Phewa Tal, but P-deficiency occurred in 100% of the experiments in Begnas Tal (Fig. 6).

Lake Taihu, China is the only other tropical/sub-tropical lake where nutrient status measurements and NSEs have been conducted throughout the year (Paerl et al., 2011; Online Appendix Tables 3 and 4; Fig. 7c). Of studies reporting NSEs during both non-summer and summer seasons, Phewa and Begnas Tal had the highest response to the addition of N and the lowest response to the addition of P, relative to controls during summer (Online Appendix Table 4). Our results match other findings in non-summer months; Phewa and Begnas Tal had the 2nd and 3rd highest response to the addition of N and P, respectively, relative to controls (Online Appendix Table 4).

In Lake Titicaca, a high-altitude tropical lake in Peru and Bolivia, N-deficiency was common until winter, when turnover brought such large quantities of N and P to the surface that neither were deficient (Vincent et al., 1984). N-deficiency was predominant during dry weather in Lake Atitlán, Guatemala, but tropical storms switched the phytoplankton to P-deficiency (Corman et al., 2015). Even alpine lakes show highly temporal patterns of phytoplankton nutrient deficiency during ice-free months from May to November (Morris & Lewis, 1988). Collectively, these results underscore the importance of incorporating year-round measurements into the N vs. P debate (Lewis & Wurtsbaugh, 2008; Paerl et al., 2016; Higgins et al., 2017).

The role of monsoons in thermal stratification

Monsoon rains impact thermal stratification in subtropical lakes (Davis et al., 1998; An & Jones, 2002; Jones et al., 2006), often expanding the mixed layer, while extreme rain events create density-dependent interflows, which have little direct influence on the epilimnion (Jones et al., 2009). Historically, interflows have been measured intermittently in Phewa Tal (Lohman et al., 1988; Davis et al., 1998; Jones et al., 2009) and their impact captured during isolated sampling events such as August 1998. During that inflow event, lake TP declined by half in 6 days (from 60 to 30 μg l^{-1}, no NSE experiments were set at this time) concurrent with a doubling of Secchi disk transparency (from 0.5 to 1 m) and a 75% decline in NVSS (from 26 to 6 mg l^{-1}, data not shown).

A sub-surface spring is a source of N to Phewa Tal with an influence that varies across seasons. In warmer months, the cool spring water descends to the metalimnion and affiliated nutrients are largely unavailable to pelagic phytoplankton, but support the sub-surface algal layer (Lohman et al., 1988; Davis et al., 1998). During winter mixis, however, N-rich spring inflow and lake temperatures are similar (~ 23°C; Fig. 2c), thereby serving as a source of N to algal communities in the photic zone.

Why does N-deficiency not dominate during the pre- and post-monsoon seasons?

Identification of P-deficiency during the holomictic pre- and post-monsoon seasons indicates that either external or internal (or both) P loading is reduced relative to during the monsoon. It could also indicate an increased supply of N during these seasons- either from external or internal sources.

Another potential component of the lake nutrient budget is internal loading (Beutel, 2006; Taylor & Nürnberg, 2009; Orihel et al., 2017). Based on seasonal patterns, we hypothesize that seasonal P-deficiency, measured during pre- and post-monsoon, was under internal rather than external nutrient control. The flux of N from lake sediments serves as an internal source of N; dissolved N forms such as ammonium accumulate in anoxic hypolimnia (Beutel, 2006; Wu et al., 2017) until circulated throughout the water column during mixis. Rai (2000) documented this pattern in both lakes during 1993–1997 (concurrent with this study). Our data showed dissolved N formed the majority of TN (80–100%) in lake water samples during post-monsoon, with minimums during the monsoon, which is consistent with internal loading. Further support by Gurung et al. (2006) shows dissolved inorganic N (DIN) in bottom water increased from June to October in Phewa Tal and was higher in surface samples. Total and dissolved N values in both lakes showed a seasonal increase during dry-season, concurrent with turnover and holomixis (Figs. 2c, 3, 4). This pattern suggests internal N loading (Présing et al., 2001; Beutel, 2006; Wu et al., 2017), and supports that internal loading of hypolimnetic ammonium contributed to the switch from N-deficiency to P-deficiency in both lakes.

Sediment release and the warm monomictic thermal pattern would likewise govern internal P loading, an important feature of the P budget in many lakes, but is also linked to iron (Fe) biogeochemistry (Orihel et al., 2017). TP concentrations were similar between surface and bottom waters during the monsoon (Gurung et al., 2006), and our data showed TP increased in Begnas during mixis (Fig. 3b), but not in Phewa (Fig. 3a). Ferric hydroxides readily formed when hypolimnetic samples from Phewa were exposed to oxygen, a phenomenon that was not similarly noticeable in Begnas samples. Greater precipitation of Fe-phosphate during mixis (Mortimer,

1941) in Phewa Tal likely accounts for this observed difference. TN and TP were correlated in Begnas but not in Phewa. In both lakes, however, TN:TP ratios increased to > 23 during turnover and subsequently declined during the pre-monsoon season (Fig. 3). In sub-tropical Australian reservoirs, high DIN:DIP (dissolved inorganic P) ratios were also observed in bottom waters during stratification providing further evidence for increased release of DIN relative to DIP from sediments (Burford et al., 2012). In our study, the proportion of TP that was dissolved did not vary among seasons regardless of stratification, suggesting that sediments were not a significant source of dissolved P to Phewa and Begnas Tal. In addition, the ratio of TN:TP reflects changing N concentrations, with P concentrations remaining relatively consistent in both total and dissolved forms across seasons.

Maximum Chl *a* concentrations occurred in post-monsoon samples, presumably in response to internal N. Our data suggest N limitation dominates in Phewa and Begnas Tal during the monsoon when nutrient loading is primarily terrestrially-derived (Downing et al., 1999; Gal et al., 2016). In the mid-hills of Nepal, agriculture plays a dominant role (Ross & Gilbert, 1999) and has intensified because of a switch from subsistence- to market-based crops (Dahal et al., 2009). This increase in agriculture increases both N and P fluxes to aquatic systems (Dahal et al., 2007), which would be diluted during the wet monsoon season (Collins & Jenkins, 1996). Both lakes are heavily influenced by siltation from agricultural practices within their watersheds (Collins & Jenkins, 1996; Ross & Gilbert, 1999; Rai, 2000). Internal processes play a more prominent role in the pre- and post-monsoon seasons, which is why they exhibit P limitation and not the hypothesized N limitation. We provide evidence that nutrient deficiencies to phytoplankton growth can switch with season, and this may be especially important to consider in monsoon-influenced sub-tropical lakes surrounded by areas of intense agriculture.

The role of light in monsoon-influenced lakes

Light plays a key role in regulating phytoplankton biomass and nutrient uptake (Falkowski & Raven, 2007; Jones et al., 2009; Dubourg et al., 2015). Chlorophyll *a*, our proxy for phytoplankton response in NSEs, is influenced by changing light conditions (Falkowski & Raven, 2007). Phytoplankton can photoacclimate to higher/lower light conditions by reducing/increasing light harvesting pigments such as Chl *a* (Arrigo et al., 2010). In our NSEs, there were no significant differences between initial Chl *a* concentrations and control values post-incubation, suggesting phytoplankton, exposed at half the Secchi depth, did not experience an appreciable change in their light environment during the experiments, nor did they exhibit indicators of light deficiency under initial conditions. We interpret the NSEs as responses to nutrient addition, independent of the complications of light limitation.

The importance of multiple scales of assessment and year-round studies

Experiments such as NSEs cannot entirely represent lake conditions, and have been criticized for limited realism (Hecky & Kilham, 1988; Carpenter, 1996). Our NSEs were supported using TN:TP as an additional indicator of nutrient deficiency to phytoplankton growth and both approaches suggested a strong seasonal component. The TN:TP ratios broadly agreed with NSE results, with two main differences. P-deficiency as determined by TN:TP ratios occurred more often than indicated by our NSEs, especially in Begnas Tal (Fig. 3). Additionally, TN:TP ratios failed to capture the N-deficiency during the monsoon, which was strongly supported by NSEs. This lack of agreement may be because seston N:P is considered more reliable to assess algal nutritional status (Healey & Hendzel, 1980), as total fractions include N and P in all forms, some of which are not readily accessible to algae. This concern may be particularly relevant to our study where the majority of total nutrients were in dissolved form (Table 1).

Conclusions

To our knowledge, this is one of few studies to comprehensively examine seasonality and nutrient deficiencies to phytoplankton growth in sub-tropical lakes over multiple years. Our data suggest we cannot apply understanding of phytoplankton nutrient deficiencies in temperate lakes to sub-tropical lakes that often experience different temperature and precipitation and thus, nutrient regimes. Flooding due to climate change is increasing globally (Döll & Zhang, 2010), which mobilizes nutrients from the watershed and increases nutrient loading to downstream water bodies, particularly when agricultural lands flood (McCullough et al., 2012). Thus, the seasonal changes in hydrology and physical lake mixing patterns could strongly influence the nutrient status of phytoplankton communities. Furthermore, light deficiencies may play an important, interacting role with the presence and type of nutrient deficiencies to phytoplankton growth (Dubourg et al., 2015). Our data underscore the complexities of lakes and emphasize the need for more research on how seasonality may influence lentic nutrient dynamics on a global scale.

Previous work suggests sub-tropical and tropical lakes tend to be N-deficient (Dussart, 1974; Jones et al., 1989; Downing et al., 1999; Abell et al., 2010). This paradigm applies during most of the year in our study lakes. We measured N-deficiency during the monsoon season, but both lakes switched to P-deficiency during holomixis. Most global lake studies are conducted during a 4–6 month period, ignoring nutrient dynamics during other seasons—especially in the sub-tropics and tropics where much of the precipitation occurs seasonally. Year-round sampling will further illustrate how seasonality affects nutrient deficiencies and thus response to nutrient management strategies in aquatic systems. We emphasize the importance of this perspective when considering the application of nutrient management strategies on a global basis.

Acknowledgements We thank Erin Petty for making Figure. 1. We also thank Jennifer Parris, Jennifer Graham, John MacEachern, Robert Bacon, Bruce Perkins, Glenn Wylie, Lance Dorsey, Prem Nepali, and IUCN Nepal for their assistance during the study. We thank Gertrud Nürnberg for advice on internal nutrient loading. This work was supported by the National Science Foundation (INT-9602105).

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