Inland Waters

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ABSTRACT

The limiting nutrient for freshwater phytoplankton productivity can vary within and between geographic regions. Understanding how local (i.e., lake and catchment) and regional (i.e., multiple catchment) factors shift the relative importance of nitrogen (N) and phosphorus (P) limitation presents a key research challenge for freshwater ecosystems and may enhance our understanding of how lakes could be managed to control eutrophication. Here, we used in situ microcosm incubations with factorial N and P amendments to determine the type of limitation in 16 lakes across the northeastern United States. Study lakes had similar climate but varied in geography, trophic status, and chemistry. Limitation by both N and P was most common (colimitation $n = 5$ and serial limitation $n = 3$); however, we also observed single nutrient N ($n = 3$) and P limitation ($n = 4$). The type of limitation was related to background phytoplankton biomass, longitude, and land use. The magnitude of response to combined nutrient enrichment was negatively related to background P concentrations. This multi-lake experiment suggests that both N and P often play a role in regulating productivity and that local and regional characteristics affect nutrient limitation patterns.

Introduction

Anthropogenic nitrogen (N) and phosphorus (P) loading in freshwater lakes can lead to cultural eutrophication, toxic cyanobacterial blooms, loss of biodiversity, and decreased property values (Kolzau et al. 2014). However, the relative importance of each macronutrient varies widely; N limitation, P limitation, serial limitation, and colimitation by N and P have all been observed in freshwater lakes (Schindler 1977, Downing and McCauley 1992, Paerl et al. 2016). Successful management of lakes depends in part upon understanding spatial variability and the direct and indirect drivers that affect nutrient limitation of phytoplankton productivity.

The paradigm that P limits phytoplankton growth in freshwater lakes formed the historical basis for policies that manage P inputs to lakes (Sterner 2008). However,

more recent studies have demonstrated the prevalence of N limitation and N + P colimitation (Abell et al. 2010, Cooper et al. 2016). At timescales (i.e., days–weeks) most relevant to the development and persistence of single algal bloom events, colimitation by N and P is especially important (Harpole et al. 2011). Despite this knowledge, no regional assessments of the variability of nutrient limitation have been conducted in northeastern North America, a region with high lake density and increasing eutrophication (Sinha et al. 2017).

Direct drivers of nutrient limitation include macronutrient concentrations and stoichiometry as well as phytoplankton community composition. Over broad regions, the resource-ratio hypothesis predicts that macronutrient stoichiometry determines patterns of nutrient limitation (Downing and McCauley 1992). For example, low ratios of total nitrogen (TN) to total phosphorus (TP)

reflect the prevalence of N limitation in New Zealand lakes (Abell et al. 2010). Using macronutrient ratios can provide a quick way of approximating lake nutrient limitation (Guildford and Hecky 2000); however, assessing nutrient limitation based solely on macronutrient ratios may include substantial error (Bergström 2010). Variation in the dominant phytoplankton taxa can also play an important role in nutrient limitation patterns because phytoplankton groups respond to nutrient enrichment differently (Mette et al. 2011). A diverse phytoplankton community may include both taxa that thrive under N limitation (e.g., N-fixing cyanobacteria) and taxa that thrive under P limitation, resulting in community-wide colimitation by both N and P (Bracken et al. 2015).

Indirect drivers of nutrient limitation are factors that modify macronutrient concentrations, stoichiometry, or phytoplankton communities, thereby affecting primary productivity. These include regional factors that create trends across multiple watersheds as well as local factors that modulate these trends within the lake or catchment. In 8 Texas reservoirs, local factors including depth and relative drainage area were more important drivers of phytoplankton productivity and N fixation rates than nutrient concentrations (Forbes et al. 2008). Large, externally driven shifts in lake pH can affect nutrient limitation status via changes in the phytoplankton community composition; at low pH the abundances of some taxa (e.g., cyanobacteria and diatoms) tend to decrease and N fixation is disrupted (Schindler et al. 1990). Finally, land use (e.g., agriculture and urbanization) and N deposition may increase lake nutrient loading and modify TN:TP ratios, thereby changing the limitation type (Arbuckle and Downing 2001, Hayes et al. 2015).

To understand how regional variation in nutrient limitation is a result of both direct and indirect drivers, we conducted standardized and synchronous experiments in lakes across the northeastern United States. We used microcosms to examine spatial variation of nutrient limitation across a broad range of lake characteristics. Our questions and hypotheses are the following: (1) How variable is nutrient limitation type within a region? (2) Do macronutrient concentrations or ratios predict nutrient limitation type and magnitude of response? (3) How do direct and indirect factors at local and regional scales relate to nutrient limitation type? We hypothesized that at time scales ecologically relevant to phytoplankton growth, colimitation is predominant across all study lakes, and nutrient limitation patterns are related to the ratio of TN to TP, as well as phytoplankton biomass and community composition.

Study sites

Our 16 focal lakes spanned 4 states in the temperate biome of the northeastern United States (Table 1, Supplemental Fig. S1). Background TN:TP and chlorophyll *a* (Chl-*a*) concentrations in the lakes ranged by an order of magnitude and spanned from oligotrophic to eutrophic (Table 1). Phytoplankton communities at the time of sampling were dominated by chlorophyta in 70% of the lakes ($n = 11$) and cyanobacteria in the remaining lakes ($n = 5$).

Methods

Microcosm incubation

In each of our 16 study lakes, we conducted an in situ incubation experiment with factorial N and P amendments (Williamson et al. 2010) to determine the macronutrient limitation of phytoplankton biomass. All experiments were deployed for 1 week starting 19–22 June 2017. At each lake, we prepared 16 ~450 mL microcosms with ~50 mL headspace (6 × 6 inch Bitran bags, Fisher Scientific, USA). We used water collected from 1 m depth and removed larger zooplankton using a 125 μm sieve. Each lake had 4 replicates of the following 4 treatments: control (no amendments), +N alone (increased nitrate [NO_3^- as $NaNO_3$] and ammonium [NH_4^+ as NH_4Cl] by 168 μg L^{-1} each above background); +P alone (increased phosphate [PO_4^{3-} as NaH_2PO_4] by 31 μg L^{-1}); and +N +P combined. We standardized amendments to ensure that nutrient addition would elicit a response from the most nutrient-rich study lake. All microcosms were randomly positioned on floating PVC racks and were covered with neutral-density filters to reduce incident light exposure by ~66%, similar to underwater light exposure (Jane and Rose 2018). Following incubations, we analyzed Chl-*a* concentration as a proxy for phytoplankton biomass using standard hot ethanol or acetone extractions in the lab closest to each lake (Supplemental Table S1).

Lake characteristics and analysis

We compiled geographic and geomorphologic data for each lake and standardized the collection and analysis of data (Chl-*a*, TP, TN, and the dominant phytoplankton phylum; hereafter, "background" variables; Table 1) from surface water before and after the incubations. Background Chl-*a* samples were analyzed using a standard spectrophotometric method with hot ethanol extraction. TN and TP samples were processed with a Lachat Quik-Chem FIA + Water Analyzer (Pritzlaff 2003). Concentrations below the method detection limit for TP and TN

Table 1. Lake characteristics, sorted by latitude (Lat), including longitude (Long), elevation above sea level (Elev), mean lake depth (Depth), surface area (SA), total nitrogen (TN), total phosphorus (TP), chlorophyll *a* (Chl-*a*), and percentage of phytoplankton cells identified as chlorophyta (Chloro) and cyanobacteria (Cyano). TN, TP, and Chl-*a* data are reported as mean values for samples collected between pre (late June) and post (early July) experiment.

Lake	State	Lat (°N)	Long (°W)	Elev (m)	Depth (m)	SA (ha)	TN (μg L^{-1})	TP (μg L^{-1})	Chl-*a* (μg L^{-1})	Chloro (%)	Cyano (%)
East	ME	44.610	69.785	80	5.5	695	280	15.7	2.7	71	1
Great	ME	44.530	69.900	76	6.4	3450	210	32.9	3.3	82	4
Long	ME	44.497	69.915	72	10.7	1035	100	9.4	3.7	58	18
Snow	ME	44.471	69.733	71	10.1	1494	200	8.8	6.7	59	20
High	VT	43.753	73.153	315	7.9	8	170	3.7	3.2	77	0
Otsego	NY	42.756	74.896	363	25.0	1637	550	7.3	9.0	58	0
Moe	NY	42.430	74.560	497	1.8	16	365	23.1	5.8	20	70
Cassadaga	NY	42.356	79.326	400	3.4	93	150	9.0	6.8	76	21
Bear	NY	42.347	79.385	399	4.6	46	250	13.0	10.4	44	34
Chautauqua	NY	42.162	79.414	399	7.6	2860	190	19.0	4.4	22	77
Mohonk	NY	41.766	74.158	379	6.0	7	190	13.6	2.7	54	18
Minnewaska	NY	41.726	74.235	503	5.7	14	160	4.3	2.8	83	0
Awosting	NY	41.706	74.290	568	5.0	39	88	3.9	1.9	70	0
Waynewood	PA	41.395	75.210	421	6.0	28	650	34.8	47.0	23	75
Lacawac	PA	41.382	75.293	439	5.2	21	100	4.5	4.8	12	86
Giles	PA	41.376	75.050	428	10.1	48	135	2.0	3.2	84	9

Note: TN includes dissolved and particulate N for all lakes except Awosting, Mohonk, and Minnewaska, whose TN was epilimnetic dissolved N collected later in the summer.

were replaced with 2 μg L^{-1} and 20 μg L^{-1}, respectively, as midpoints between 0 and the detection limit. We determined phytoplankton community composition by counting >200 individuals and identifying to phylum using the Utermöhl method (Lund et al. 1958).

Determination of nutrient limitation

For each lake, we performed a 2-way ANOVA to analyze the change in Chl-*a* with N or P amendments (presence/absence) as factors using the *cars* R package (Fox and Weisberg 2011). All statistical analyses were conducted in R software (R Core Team 2017). If Chl-*a* values across interaction treatment levels had heterogeneous variances (Bartlett test, $p < 0.05$), we log-transformed data prior to running the ANOVA (50% of the lakes). Based on the interaction and main effects, we determined if there was no nutrient limitation, single factor limitation, serial limitation, or colimitation (Supplemental Table S2). Single-factor limitation was defined as a significant effect of one nutrient, with neither a significant effect of the other nutrient nor a significant interaction term. Serial limitation was defined as a main effect of one nutrient along with a significant interaction between the 2 nutrients. Colimitation included cases with a significant interaction term and either no main effects or both main effects (simultaneous colimitation), and cases when both nutrients enhanced productivity but there was no interaction (independent colimitation; Harpole et al. 2011).

Effect size analysis

We calculated the effect size for the N and P treatments (RR$_{N}$ and RR$_{P}$) as the ratio between the mean Chl-*a*

concentration from treatments containing the nutrient of interest (N or P) relative to the mean of the other single nutrient treatment and the control (C; equations 1 and 2). We calculated the combined N and P effect size (RR$_{NP}$) as the ratio between the mean Chl-*a* concentration from the combined nitrogen and phosphorus (NP) treatment and the sum of the mean Chl-*a* concentrations from N, P, and C treatments with a correction for the number of means being compared (equation 3). RR$_{NP}$ represents the colimitation effect size and is predominantly indicative of simultaneous colimitation.

$$\text{RR}_\text{N} = \left(\frac{\bar{\text{N}} + \overline{\text{NP}}}{\bar{\text{C}} + \bar{\text{P}}} \right) \qquad (1)$$

$$\text{RR}_\text{P} = \left(\frac{\bar{\text{P}} + \overline{\text{NP}}}{\bar{\text{C}} + \bar{\text{N}}} \right) \qquad (2)$$

$$\text{RR}_\text{NP} = \left(\frac{3\overline{\text{NP}}}{\bar{\text{C}} + \bar{\text{N}} + \bar{\text{P}}} \right) \qquad (3)$$

We used linear regressions to test the relationship between background TN:TP ratios and the phytoplankton response to N relative to P (RR$_{N}$/RR$_{P}$), as well as the relationships between the background TN concentrations, TP concentrations, TN:TP ratios, and the NP response ratio. Because of nonnormality (Shapiro–Wilk test, $p < 0.05$), we natural-log transformed explanatory variables.

Drivers of limitation type

To test whether TN:TP ratios accurately classified the limiting nutrient, we plotted the nutrient limitation types on a biplot with delineated regions of hypothesized

N or P limitation using TN:TP molar ratios that indicate P limitation (>110) and N limitation (<44) for lake phytoplankton from Elser et al. (2009). Considerable uncertainty surrounds the appropriate threshold ratio, in part because TN contains a highly variable amount of recalcitrant total dissolved nitrogen (TDN). For our analysis, we chose delineations from Elser et al. (2009) rather than the commonly cited values from Guildford and Hecky (2000; N-limitation cut-off = 20 and P-limitation cut-off = 50) because Guildford and Hecky's ratios are based on a small set of oceans and lakes and may underestimate N limitation in freshwater systems (Bergström 2010). By contrast, Elser et al. (2009) suggested thresholds based on an analysis of nutrient limitation in many waterbodies similar to the lakes from this study.

To determine potential external drivers of nutrient limitation type, we ran a regression tree analysis with the R package *partykit* (Hothorn and Zeileis 2015). We constructed the tree with geography (longitude, elevation), land use (percent agriculture and developed land), geomorphometry (max depth, surface area), and chemistry (background pH) as indirect explanatory variables (Table 1) using the *anova* method. Because the number of lakes in our study was limited, we used this approach as an exploratory analysis while being conservative and using the one standard deviation rule to prune the overfit tree (Knoll et al. 2015). We compared background Chl-*a* concentrations across the regression tree divisions using a Kruskal–Wallis nonparametric ANOVA because of small sample size and nonnormality with a post hoc Dunn test for means comparisons (R package *dunn.test*; Dinno 2017).

Results

Nutrient limitation

Phytoplankton biomass was limited by P alone in 25% of the lakes ($n = 4$) and limited by N alone in 19% ($n = 3$). In half of the study lakes ($n = 8$), phytoplankton biomass was either colimited or serially limited by both N and P (Fig. 1). Of those lakes, 2 had serial P limitation and 1 had serial N limitation. In 1 lake, phytoplankton biomass was not limited by N, P, or their combination (Fig. 1).

Effect size analysis

The effect size of N relative to P was significantly related to the TN:TP ratio in each lake ($\ln(RR_N/RR_P) = 2.74 - 0.8 \times \ln(TN:TP)$, $p = 0.02$, $R^2 = 0.29$) and delineates P-limited lakes from N-limited lakes (Fig. 2b). P-limited lakes had a response ratio <1, indicating P limitation, whereas N-limited lakes all had response ratios

indicative of N limitation ($RR_N/RR_P > 1$). Colimited and serially limited lakes were both scattered across the full range of TN:TP ratios but followed the same trend (Fig. 2b). The RR_{NP} was negatively related to the background concentration of TP ($RR_{NP} = 6.2 - 1.2 \times \ln(TP)$, $p = 0.03$, $R^2 = 0.23$; Fig. 2c). The RR_{NP} was higher in colimited and serially limited lakes (median = 4.1) relative to the other lakes (median = 2.1); however, no relationship was found between the RR_{NP} and background TN concentrations or background TN:TP ($p > 0.05$; Fig. 2d).

Drivers of limitation type

All of the N-limited lakes fell below the TN:TP threshold (TN:TP = 44; Elser et al. 2009) for N limitation (Fig. 2a). Three colimited lakes, 1 serial N-limited lake, and 2 P-limited lakes also fell in that range (Fig. 2a). Only 2 lakes (1 P limited and 1 colimited) were above the TN:TP ratio delineating P limitation (TN:TP = 110; Elser et al. 2009). One colimited lake, 2 serial P-limited lakes, 1 P-limited lake, and the lake with no observed limitation were in the middle range predicting colimitation (Fig. 2a). Longitude and land use were the 2 indirect factors that best explained patterns of nutrient limitation in the conservative regression tree model. The first split in the final regression tree was based on longitude; the 3 lakes that were farthest west (>77.3°W) were all N limited (Fig. 3). The eastern lakes were then divided based on anthropogenic land use; the 3 lakes with the most agriculture, residential, and urban lands (>15.8%) were all P limited (Fig. 3). The remaining lakes included all serial and colimited lakes, 1 P-limited lake, and 1 lake with no nutrient limitation observed. Chl-*a* concentration varied significantly across the 3 categories identified by the regression tree ($\chi^2 = 9.4$, df = 2, $p = 0.01$). The western (9.0 µg L^{-1}; median) and eastern (6.8 µg L^{-1}; median) lakes, both with higher anthropogenic land use in their catchments, were statistically similar and had significantly higher chlorophyll than the rest of the lakes (3.2 µg L^{-1}; median).

Discussion

Our results demonstrate a high prevalence of colimitation and serial limitation as well as roughly equal frequency of single nutrient N or P limitation across a regional suite of lakes in the northeastern United States. Prior evidence for limitation by both N and P (Paerl et al. 2016) and responses to single N and P additions (Elser et al. 1990) are broadly supported. Our results revealed the same general patterns as a meta-analysis across 62 lakes (Elser et al. 1990) that found similar proportions of N and P limitation. Using standardized experiments,
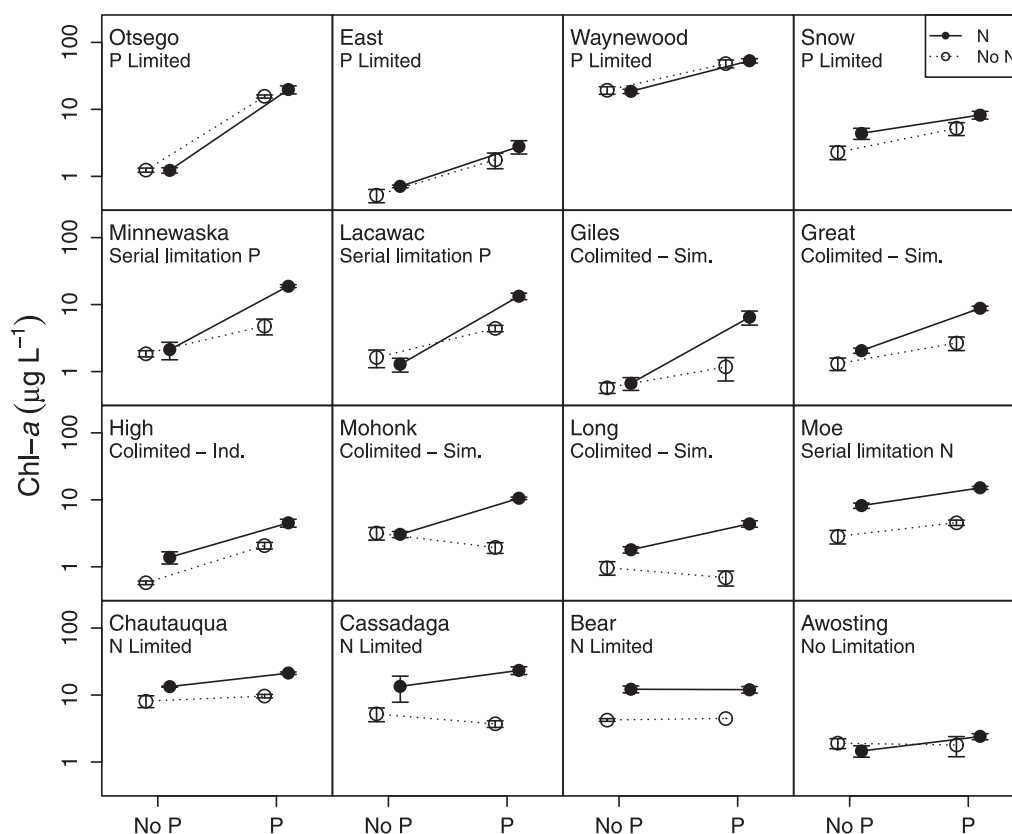
Figure 1. Interaction plots of Chl-*a* response to nutrient addition in 16 lakes. The x-axis indicates whether or not P was added. N and no N additions are depicted as a solid line and a dotted line, respectively. Lake name and limitation type are indicated at top left of each panel including serial limitation with the primary nutrient identified (S_P or S_N). Colimitation is broken into simultaneous (sim.) and independent (ind.). Panels are arranged by limitation type. Error bars represent standard error for each treatment.

we found that both regional and local lake-specific drivers explained the observed variation in nutrient limitation types.

In half the lakes we noted a synergistic increase in productivity suggesting limitation from both N and P (Fig. 1), an increasingly common occurrence in freshwater lakes (Elser et al. 2009). The prevalence of colimitation and serial limitation could be facilitated by 2 mechanisms that are not mutually exclusive. First, additional nutrients of one type could allow individual phytoplankton cells to access other previously unavailable nutrients. For example, additional N may allow the production of N-rich enzymes (e.g., phosphatase) that facilitate access to P (Bracken et al. 2015). Second, colimitation can be facilitated at the community level when various taxa are limited by different nutrients. This process is most clearly seen in High Pond, which had independent colimitation with both N and P main effects but no significant interaction (Table 2). In this lake, part of the phytoplankton community was likely N limited while another part was P limited, with combined additive effects. Colimitation at the community level may be common in lakes comprising diverse

phytoplankton communities, especially those that include N-fixing taxa (Harpole et al. 2011). In our study, colimitation was not seen consistently within the predicted range of the resource-ratio hypothesis (Fig. 2a; Elser et al. 2009) and was clustered in eastern lakes with lower background phytoplankton biomass (Fig. 3). Phytoplankton diversity is greatest at low to intermediate phytoplankton biomass (Stomp et al. 2011), which may lead to increased coexistence of taxa that favor either higher or lower TN:TP in these lakes. Diverse taxa, low overall phytoplankton biomass, and low bioavailability of nutrients might contribute to fine-scale serial limitation. This result would appear as colimitation where as soon as one nutrient is increased slightly, the other rapidly becomes limiting.

We detected N limitation in 3 lakes, an expected finding when P concentrations are high, allowing additional N to increase phytoplankton biomass (Baker et al. 2018). During cyanobacterial blooms, phytoplankton biomass can increase faster than ammonium is regenerated, leading to high rates of N demand and N limitation of blooms (Gardner et al. 2017). Therefore, the occurrence of N limitation is possible when N
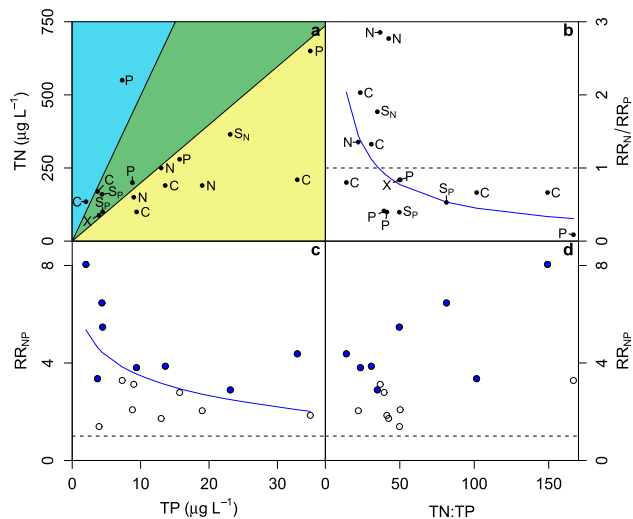
Figure 2. Effects of in-lake nutrient concentrations on the type and strength of nutrient limitation, where C is colimited, S_P and S_N are serial limitation with primary P and N limitation, respectively, N is N limited, P is P limited, and X is no macronutrient limitation. (a) A test of the resource-ratio hypothesis following Burson et al. (2018; Fig. 1a), with TN:TP ratios typical of N limitation (yellow), colimitation (green), and P limitation (blue, top section). (b) The phytoplankton response of N (RR_N) relative to P (RR_P) compared to the TN:TP ratio. The dashed line indicates a proportional response to N and P. The NP (RR_{NP}) effect size relative to (c) TP concentration and (d) TN:TP. Filled circles are lakes with colimitation and serial limitation, and open circles are remaining limitation types. Dashed line represents 1 (i.e., no treatment effect).

fixation does not satisfy phytoplankton nutrient demands. In this study, phytoplankton community composition, measured as phyla diversity, was not a significant predictive factor for nutrient limitation type. We found no increase of cyanobacteria in lakes with low TN:TP ratios, suggesting N fixation was not occurring at high rates in these lakes at the time of our experiment.

All observed instances of N limitation fell within the range of TN:TP ratios that would predict N limitation
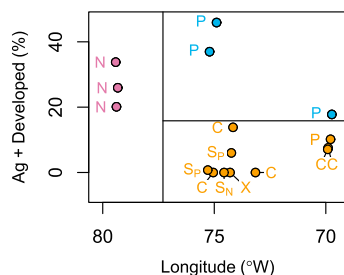

Figure 3. Regression tree breakdown of nutrient limitation type by longitude first and then agricultural, residential, and urban land cover (Ag + Developed percentage). C is colimited, S_P and SN are serial limitation with primary P and N limitation, respectively, N is N limited, P is P limited, and X is no macronutrient limitation.

(TN:TP < 44; Elser et al. 2009; Fig. 2a). However, P limitation and colimitation were not entirely explained by nutrient ratios, countering the resource-ratio hypothesis (e.g., Downing and McCauley 1992, Burson et al. 2018; Fig. 2a). Other studies have delineated different stoichiometric boundaries for limitation types. For example, Guildford and Hecky (2000) gave a TN:TP threshold of 50 for P limitation, which would increase the number of P-limited lakes correctly identified by TN:TP in our study but would underestimate N limitation. This result is consistent with Bergström (2010), who found that the values from Guildford and Hecky (2000) underestimated N limitation in oligotrophic lakes.

Longitude was most important in explaining observed patterns of nutrient limitation among our study lakes, where the 3 most western lakes were all N limited (Fig. 3). These western lakes had the highest watershed agricultural land use among our study lakes (Fig. 3). Dairy farming, which can dramatically increase nutrient fluxes, is common in these 3 watersheds (Howarth et al. 1996). High P loading from agricultural land and N recycling rates too slow to keep up with ammonium demand during periods of high productivity (Gardner et al. 2017) may be causing the observed N limitation. Therefore, nutrient stoichiometry, as influenced by land use, N speciation, and recycling, may be important in determining limitation type (Harpole et al. 2011).

Similar to the N-limited lakes, P-limited lakes had catchments >15% dominated by agriculture, urban, and residential lands (Fig. 3). However, P-limited lakes had higher phytoplankton biomass and greater TN:TP ratios than the N-limited lakes (Fig. 2a). Agriculture can affect nutrient stoichiometry, but the direction of the response depends on the type of agriculture: animal-based agriculture generates low TN:TP loads while row crops generate higher TN:TP ratios (Arbuckle and Downing 2001). In this study, we did not differentiate between animal- and crop-based agriculture. These P-limited lakes likely had an established and growing phytoplankton community at the time of the experiment, as evidenced by high early season productivity (Table 1). Early summer P limitation in productive lakes is consistent across many systems, with lakes often shifting to colimitation or N limitation as summer progresses (Kolzau et al. 2014).

Phytoplankton biomass increased during nutrient additions in microcosms from all of our study lakes except for Awosting. This lake had a pH <6 and a history of acidification (Richardson et al. 2018). Phytoplankton in Awosting could be constrained by pH directly through loss of acid-sensitive and N-fixing phytoplankton (Findlay 2003) or possibly by low concentrations of micronutrients (e.g., Ca^{2+}; Richardson et al. 2018).

Table 2. Two-way ANOVA F statistics and *p*-values for N and P main effects and interactions for each lake and resulting classification of nutrient limitation.

Lake	Main effect for N: *p*-value	Main effect for P: *p*-value	Interaction effect for N+P: *p*-value	Interpretation
Otsego	0.928	**<0.001**	0.203	P limited
East	0.197	**0.002**	0.928	P limited
Waynewood	0.905	**<0.001**	0.499	P limited
Snow	0.136	**0.047**	0.616	P limited
Minnewaska	0.828	**0.030**	**<0.001**	Serially limited – S_P
Lacawac	0.678	**0.005**	**0.022**	Serially limited – S_P
Giles	0.817	0.454	**0.018**	Colimited – simultaneous
Great	0.293	0.067	**<0.001**	Colimited – simultaneous
High	**0.007**	**<0.001**	0.976	Colimited – independent
Mohonk	0.834	0.084	**<0.001**	Colimited – simultaneous
Long	0.071	0.515	**<0.001**	Colimited – simultaneous
Moe	**<0.001**	0.090	**0.002**	Serially limited – S_N
Chautauqua	**0.010**	0.209	0.450	N limited
Cassadaga	**0.041**	0.539	0.086	N limited
Bear	**<0.001**	0.619	0.710	N limited
Awosting	0.432	0.844	0.201	None

Note: Bold *p* values indicate significant effects ($p < 0.05$). S_N and S_P indicate serial limitation with N and P as the primary limiting nutrient, respectively.

The nutrient limitation effect size is an important measurement of the magnitude of a response to each nutrient addition. Because we added the same concentration of nutrients to each microcosm, lakes with higher background nutrient concentrations experienced a lower proportional change in concentration, which was greater in TP (2–17 fold increases) than TN (1.5–5 fold increases). Therefore, the negative relationship between TP and RR_{NP} (Fig. 2c) likely indicates that the change in phytoplankton biomass is related to the proportional change in nutrient concentrations. The colimited lakes span the range of TP and TN values as well as TN:TP; colimitation is not restricted based on background nutrient concentrations.

The results presented here can be used to understand lake ecosystems; however, we acknowledge limitations of upscaling microcosm experiments. When studying nutrient limitation, it is valuable to observe the response to manipulation at the ecosystem scale (Schindler et al. 2008). However, whole-lake experiments are expensive, labor-intensive, and difficult to replicate. Microcosms are limited by oversimplification of biological communities via exclusion of zooplankton and fish, loss of interaction with the atmosphere and sediments, and omission of natural mixing patterns (Dzialowski et al. 2014). Still, microcosms are invaluable because they can be easily replicated in factorial treatment designs to test hypotheses and give measurements of nutrient limitation at broad spatial and fine temporal scales (Fraser and Keddy 1997, Sterner 2008). In light of this, our methods and results allow comparison of nutrient limitation patterns of pelagic phytoplankton communities in early summer across lakes ranging from oligotrophic to eutrophic. These results help explain the diversity of nutrient limitation in lakes at a time scale relevant to seasonal phytoplankton production.

In this study, we found high spatial variability in lake nutrient limitation of freshwater phytoplankton across the northeastern United States. Our results indicate that both N and P are important drivers of phytoplankton dynamics at short time scales, and that in-lake and catchment characteristics play an important role in determining nutrient limitation status. Given this documented diversity in nutrient limitation patterns, management may need to be tailored to specific lakes rather than implemented at state or regional scales, especially when responding to short-term phytoplankton growth.

Disclosure statement

No potential conflict of interest was reported by the authors.

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